THESIS

submitted to

THE UNIVERSITY OF GLASGOW

in fulfilment of the
requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

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August, 1954.

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ACKNOWLEDGEMENTS

The author wishes to thank Professor F. S. Spring, F.R.S., for his continued interest and encouragement, and Dr. H. R. Bentley for many practical suggestions during the course of this work.

Chemistry of

cycloArtenol and cycloLaudenol

CONTENTS

HISTORICAL

Introduction.

The triterpenes have been defined as naturally occurring compounds containing thirty carbon atoms, which can theoretically be built up of isoprene units linked regularly or irregularly. The majority of these triterpenes have a five-ring carbocyclic skeleton. There are also the tricyclic ambrein and the aliphatic squalene. Mono- and dicyclic triterpenes are unknown.

However, it has recently been shown that some compounds assigned to the small but increasing group of tetracyclic triterpenes do not conform to either of the criteria of the classical definition given above. None of the compounds of the lanosterol series can be built up according to the isoprene rule and some members of the series have recently been shown to contain thirty-one carbon atoms. It may be argued that lanosterol, for instance, should not be considered as a triterpene at all, but as a trimethyl sterol. Alternatively, a wider definition might be considered which would include the classical triterpenes, the lanosterol series and the sterols.

The work described in this thesis was concerned only with the structures of compounds related to lanosterol.

It is not, therefore, proposed to discuss in this historical review the chemistry of the large class of pentacyclic triterpenes made up by the β-amyrin group, the α-amyrin group and the lupeol group.

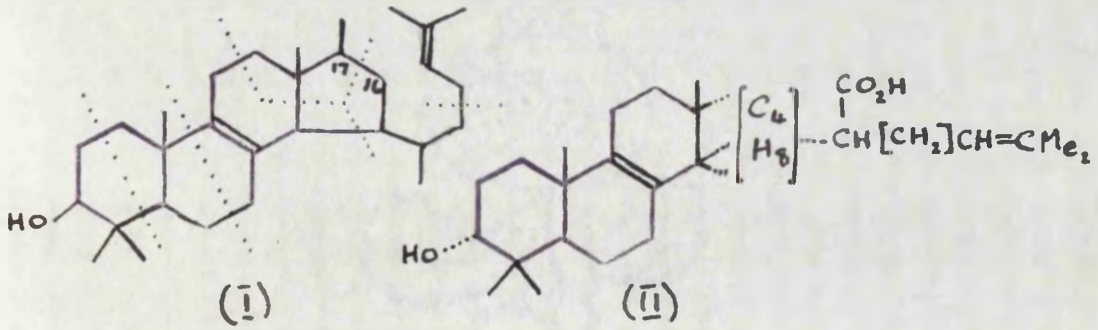
All the tetracyclic triterpenes whose basic structures are known, have been shown to have the perhydrocyclopentenophenanthrene ring system found in steroids. Recently Dawson, Halsall and Swayne (1) have stated that present evidence allows the established tetracyclic triterpenes to be divided into two groups, one typified by lanosterol and the other by euphol. This evidence includes the molecular rotation change caused by dehydrogenation at two of the carbon atoms at positions α to the usual tetrasubstituted double bond. In the lanosterol series, formation of the diene is accompanied by a positive change of molecular rotation, whereas in the euphol series a very large negative change results. There are characteristic differences between the two series in the ultra-violet absorption spectra of the dienes. Also, the products obtained by acid rearrangement of the nuclear double bond in a compound indicate the series.

Classification of the tetracyclic triterpenes.

(1) The euphol group consists of euphol, α-elemolic acid, β-elemonic acid, euphorbol and tirucallol.

Euphol is isolated from the latex of various Euphorbiaceae and was first found by Newbold and Spring (2). Formula (I) represents the structure recently suggested by Ruzicka (3). The only point in doubt is the site of the methyl group which is either on $C_{(18)}$ or $C_{(17)}$. Evidence distinguishing between the two possibilities has not yet been published. Structure (I) may be divided into six isoprene units as shown.

α-Elemolic and β-elemonic acids are isolated from Manila elemi resin. Ruzicka (4) characterised the first as a diethenoid, tetracyclic, monohydroxy-acid, $C_{30}H_{48}O_3$, and the latter as the corresponding ketone. Halsall, Meakins and Swayne (5), in the most recent publication on the subject, give partial structure (II) for α-elemolic acid.



(I) (II)

Euphorbol is found together with euphol in the Euphorbiaceae (2). At present it is known (6) that the compound is a tetracyclic, doubly unsaturated, secondary alcohol, $C_{30}H_{50}O$. One double bond is unreactive and analogous to that in the euphol nucleus; the other is present as a reactive vinylidene group.

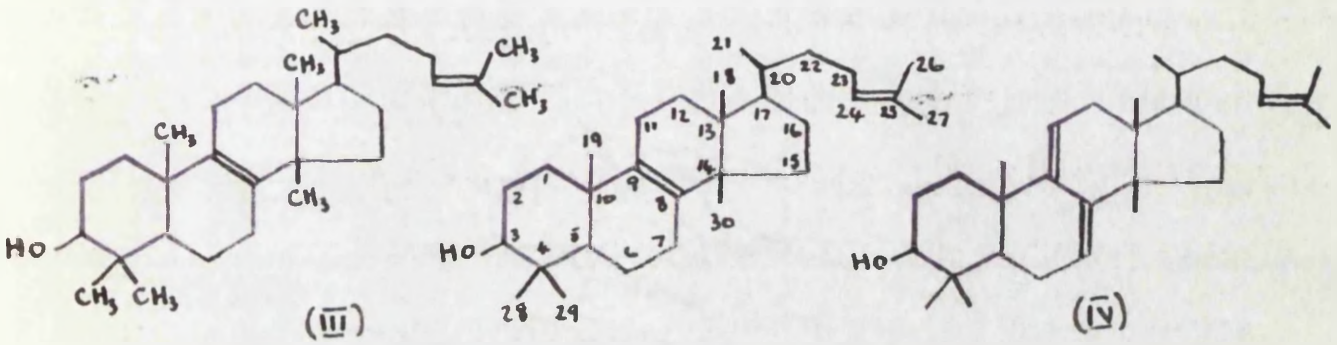
Tirucallol was isolated from Euphorbia tirucalli L. by Haines and Warren (7). Barbour, Bennet and Warren (3) have characterised the compound as a tetracyclic, doubly unsaturated, secondary alcohol, $C_{30}H_{50}O$. It has an unreactive double bond and a reactive one contained in an isopropylidene group.

(ii) The lanosterol group of naturally occurring compounds consists of lanosterol, dihydrolanosterol, agnosterol, dihydroagnosterol, the polyporenic acids A, B and C, eburicoic acid and butyrospermol. Of these, the first four contain thirty carbon atoms while the polyporenic acids and eburicoic acid have thirty-one carbon atoms. As yet, little is known about butyrospermol.

Lanosterol (alnosta-8:24-dien-3-ol) was first isolated from "isocholesterol", obtained from the neutral fraction of sheep wool grease by Windaus (9). Formula

(III) represents the known structure of lanostadienol.

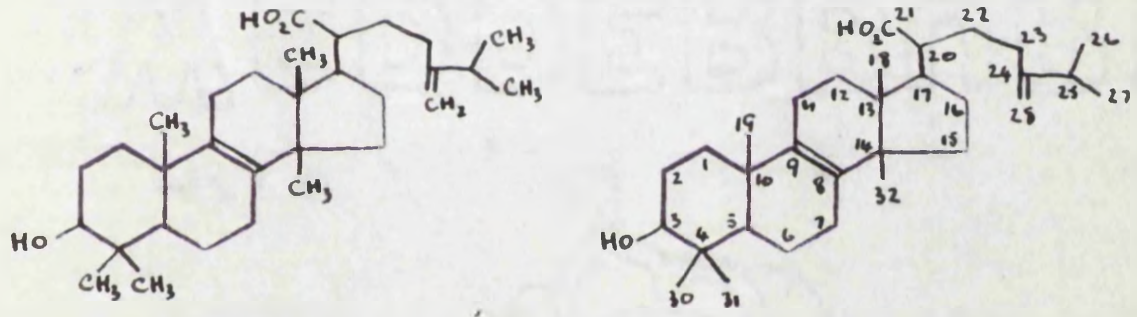
Agnosterol (lanosta-7:9(11):24-trien-3-ol) was also isolated from "isocholesterol" by Windaus (9). The established structure is as in formula (IV).



Dihydrolanosterol and dihydroagnosterol were found in "isocholesterol" by Ruzicka (10). They may be prepared by hydrogenation of the side chain double bond of lanosterol and agnosterol respectively. Ruzicka (10) showed that lanosterol and dihydrolanosterol form 50% of "isocholesterol", dihydroagnosterol 20%, while agnosterol is only present in small amounts.

Eburicoic acid was first isolated by Japanese workers (11) from the mycelium of the fungus Fomes officinalis Fr. Subsequent investigations by Robertson and co-workers (12) have shown that eburicoic acid may be isolated from five species of fungi belonging to the class Basidiomycetes. They have also shown that in two

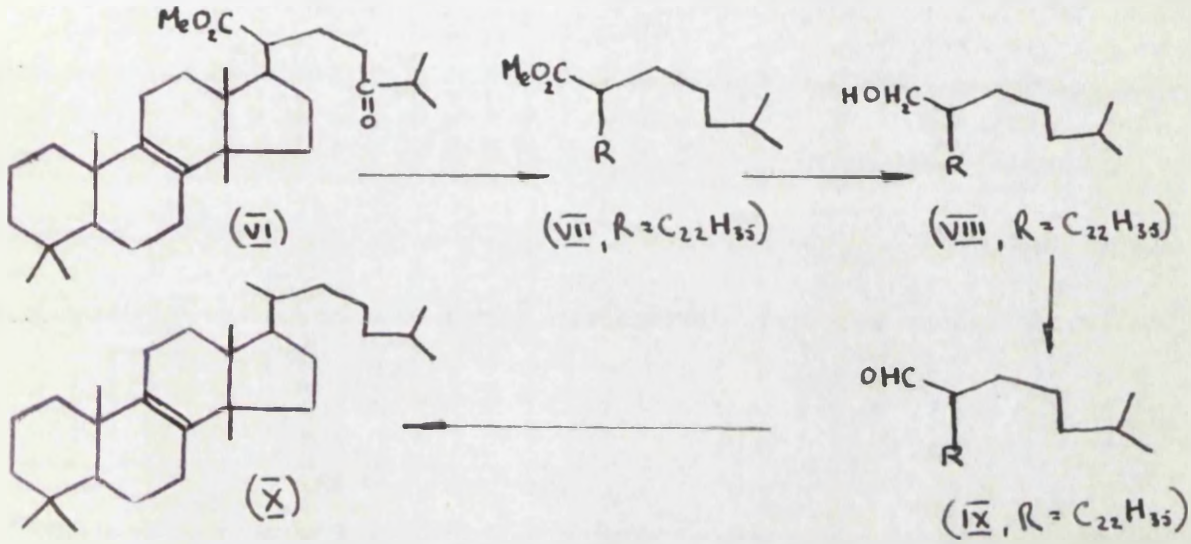
species, Lentinus dactyloides Clel. and Fomes officinalis Fr., eburicoic is accompanied by a conjugated diene-acid, dehydro-eburicoic acid, which bears to it the same relationship as does agnosterol to lanosterol. Robertson has assigned eburicoic acid, which is $C_{31}H_{50}O_3$, the structure represented by formula (V). In numbering the carbon atoms of the structure the number 29 has been reserved for use in the, as yet unknown, compounds having the side chain found in stigmasterol.



(V)

Robertson's elegant work on eburicoic acid was concluded by the conversion of eburicoic acid to lanost-8-ene (X) (12). Eburicoic acid was converted in several stages to methyl 24-oxo-28-noreburic-8-en-21-oate (VI). Wolff--Kishner reduction of (VI) followed by esterification gave methyl 28-noreburic-8-en-21-oate (VII) which was reduced by lithium aluminium hydride to the primary alcohol (VIII). Oppenauer oxidation of (VIII) gave

23-noreburic-8-en-21-ol (IX) which was reduced by the Wolff-Kishner procedure to 23-noreburic-8-ene. This was shown to be identical with lanost-8-ene (X).

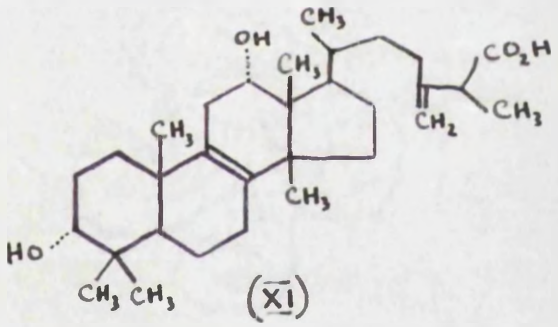


There is conclusive evidence that the configuration of the hydroxyl group at $C_{(3)}$ is identical for eburicoic acid and lanosterol. It is also regarded as unlikely that any stereochemical rearrangement occurs in the conversion of eburicoic acid to lanost-8-ene and, therefore, the stereochemistry of eburicane is the same as that of lanostane.

The polyporenic acids A, B and C were first isolated from the birch tree fungus, Polyporus betulinis Fr. by Cross, Elliot, Heilbron and Jones (13). In a later communication, Birkinshaw, Morgan and Findlay (14) describe

the isolation from the wood-rotting fungus Polyporus benzoinus (Wahl.) Fr. of an acid which has since been shown to be identical with polyporenic acid C.

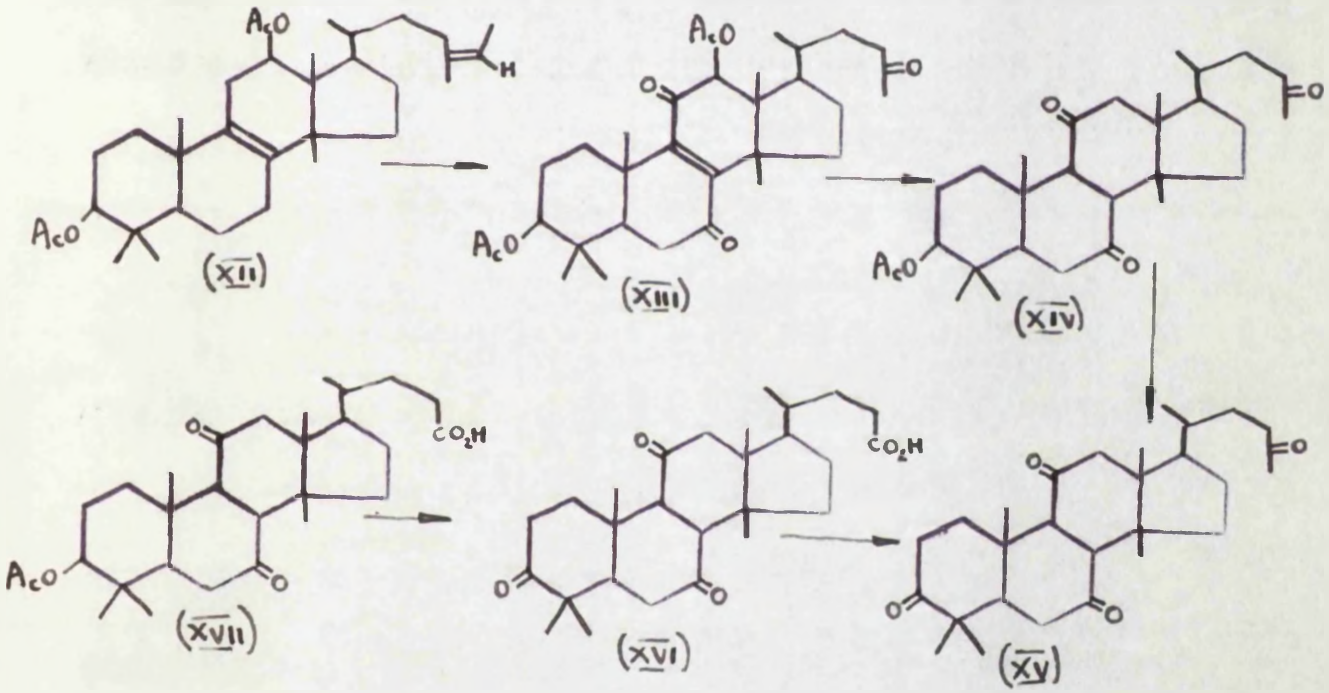
Polyporenic acid A, $C_{31}H_{50}O_4$, has been characterised as a diethenoid dihydroxy-monocarboxylic acid. The structure has been elucidated largely by work of Jones and co-workers reported in a recent series of papers (15-18). As a result of exhaustive degradative work, Jones (18) provisionally suggested that the acid has the eburicane skeleton and is 3α:12α-dihydroxyeburico--8:24(28)-dien-26-oic acid (XI).



(XI)

Jeger (19) has since confirmed this structure by converting lanostadienol and polyporenic acid A to a common intermediate (XV). Decarboxylation of polyporenic acid diacetate by heating to the melting point gave (XII) which on chromic acid oxidation gave (XIII). Treatment with zinc in acetic acid removed the acetoxy group and

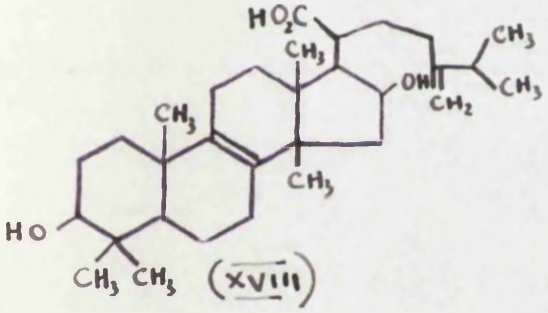
reduced the double bond to give (XIV). The tetra-ketone (XV) was formed by oxidation of the 3-hydroxy compound obtained from (XIV). Lanostadienol was converted to trisnorlanosta-7:11-dioxo-3-acetoxy-24-oic acid (XVII) in three stages. (XVII) was hydrolysed and oxidised to the 3-ketone (XVI). (XV) was prepared by treatment of the acid chloride of (XVI) with dimethylcadmium.

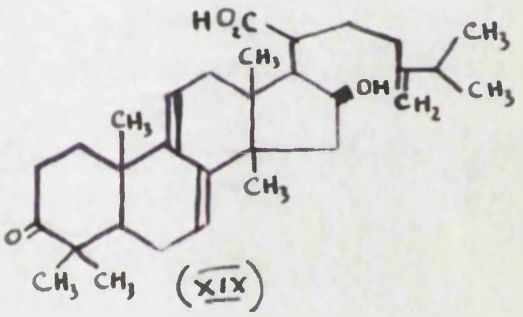


Polyporenic acid B which is isomeric with A is stated by Jeger (19) on evidence of Jones and Halsall (''The constituents of the wood-rotting fungus Polyporus betulinis'', XIIIth International Congress of Pure and Applied Chemistry, Stockholm, 29th July, 1953)

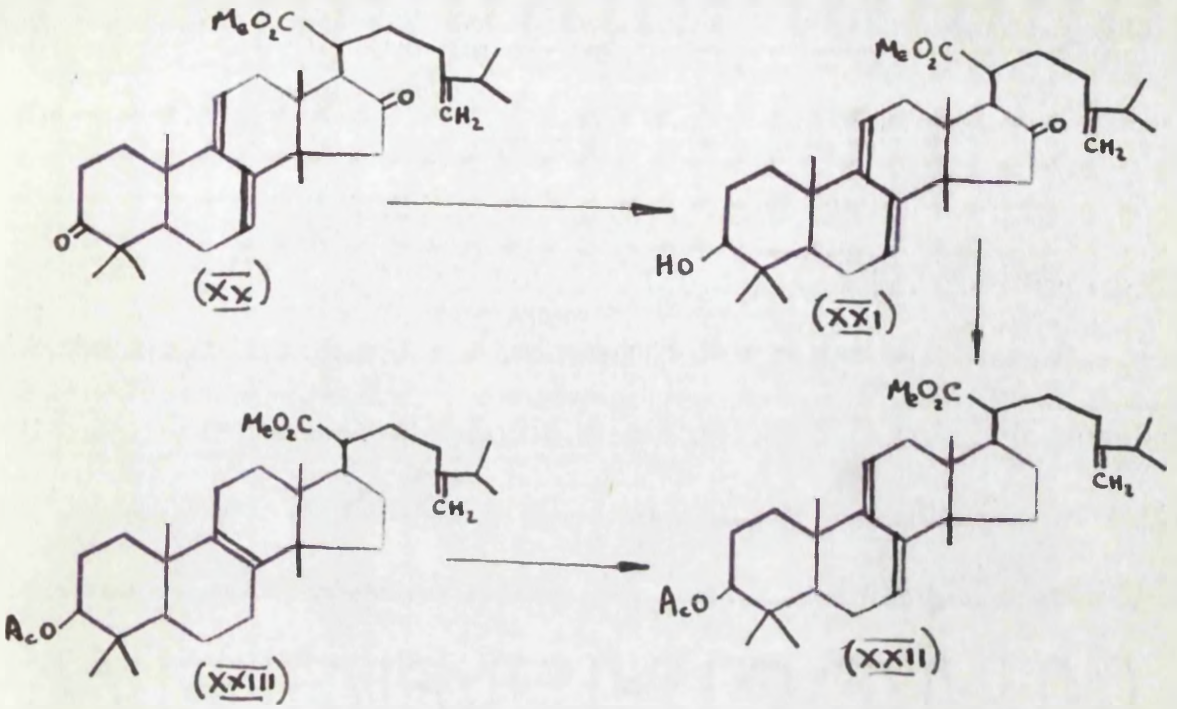
to have a structure represented by formula (XVIII). This evidence has not as yet been published.

Polyporenic acid C, $C_{31}H_{48}O_4$, has been characterised and assigned a structure by Jones and his co-workers (20); it is a triethenoid tetracyclic hydroxy-keto-carboxylic acid. Jones gives the structure as (XIX) and establishes this by conversion into a derivative of eburicoic acid which, as shown above, has in turn been converted by Robertson (12) into a lanosterol derivative.





Oxidation of methyl polyporenate C with chromic acid gave the diketo-methyl ester (XX) which was reduced by sodium borohydride to (XXI). Wolff-Kishner reduction of (XXI) followed by methylation and acetylation gave methyl acetyl dehydroeburicoate (XXII) identical with the product obtained by selenium dioxide oxidation (21) of methyl acetyl eburicoate (XXIII).



It is assumed that no stereochemical inversion occurs during the above reactions and that, therefore, the parent hydrocarbon of polyporenic acid C is eburicane which has the same stereochemistry as lanostane.

Evidence as to the configuration of the $C_{(16)}$-hydroxyl group is not complete but the reactions investigated are best explained by assuming β-configuration.

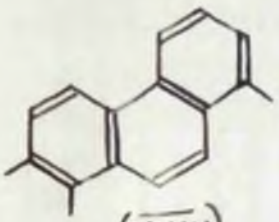
Butyrospermol has been isolated from shea nut fat by Heilbron, Jones and Robins (22) and also by Seitz and Jeger (23). The compound has been characterised as a tetracyclic, diethenoid, secondary alcohol, probably

$C_{30}H_{50}O$. The unreactive double bond appears to be (24, 25) in a similar cyclic position to that in lanosterol. The reactive double bond is contained in an isopropylidene group (22,23).

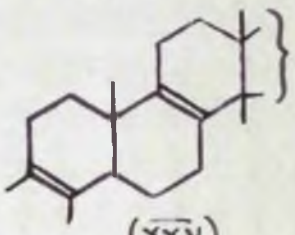
Dehydrogenation Products.

The technique of dehydrogenation by pyrolysis with selenium which was developed in the steroid and pentacyclic triterpene fields has proved valuable when applied to the tetracyclic triterpenes. These compounds have not given the confusing variety of degradation products that was obtained from pentacyclic sources, and the products correspond to larger fragments of the original molecules.

Schulze in 1936 (26) first identified the main dehydrogenation product of lanostadienol as 1:2:8-trimethylphenanthrene (XXIV), thus accounting for three rings in the structure. Ruzicka (27) obtained the same product, among others not identified, from ''isocholesterol''. On the basis of later knowledge of the formula of lanosta-

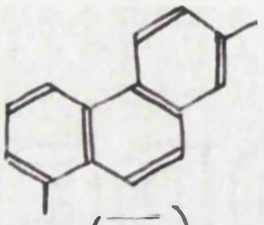


(XXIV)



(XXV)

dienol, Ruzicka (23) interpreted this as the result of a retropinacoline rearrangement of ring A in the sense of (XXV) to give (XXIV).

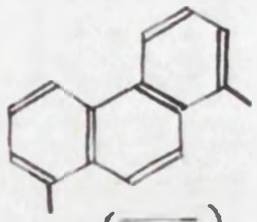


(XXVI)

Barton (29) dehydrogenated ''lanostene'' (prepared from ''isocholesterol'' and containing lanost-8-ene and lanosta-7:9(11)-diene) and ''lanosterol'' (also from ''isocholesterol'' and containing lanost-8-enol and lanost-7:9(11)-dienol) and obtained (XXIV) from both. The fact that (XXIV) was isolated in considerably better yield from ''lanostene'' than from ''lanosterol'' appears to disprove the above theory and to show that the phenanthrene ring containing the 1:2-dimethyl grouping corresponds not to ring A but to ring C in lanosterol. (XXIV) has also been obtained by dehydrogenation of polyporenic acid A (17).

In the euphol group, Jeger (30) has shown that eupha-8:24-diene gives 1:2:8-trimethyl- (XXIV), 1:7-dimethyl-

(XXVI) and 1:8-dimethyl-phenanthrene (XXVII).



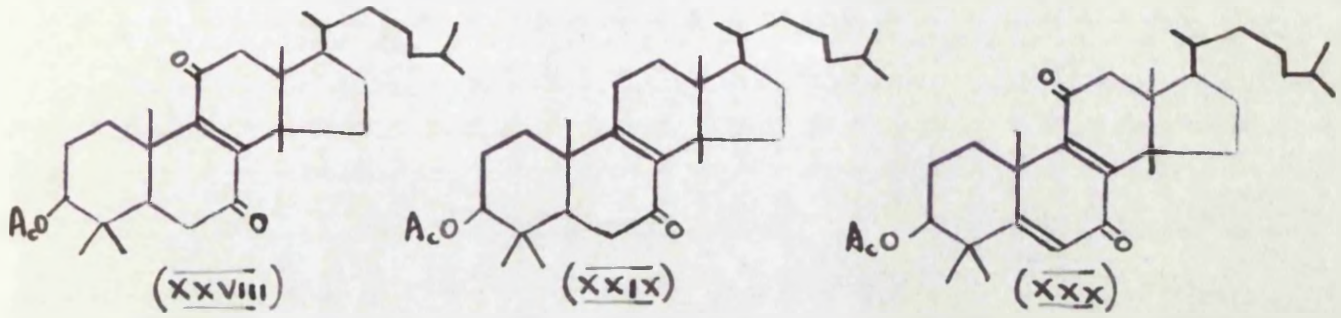
(XXVII)

(XXIV) and (XXVI) have been obtained from α-elemolic acid (31) together with other unidentified products.

Oxidations in the Lanosterol Nucleus.

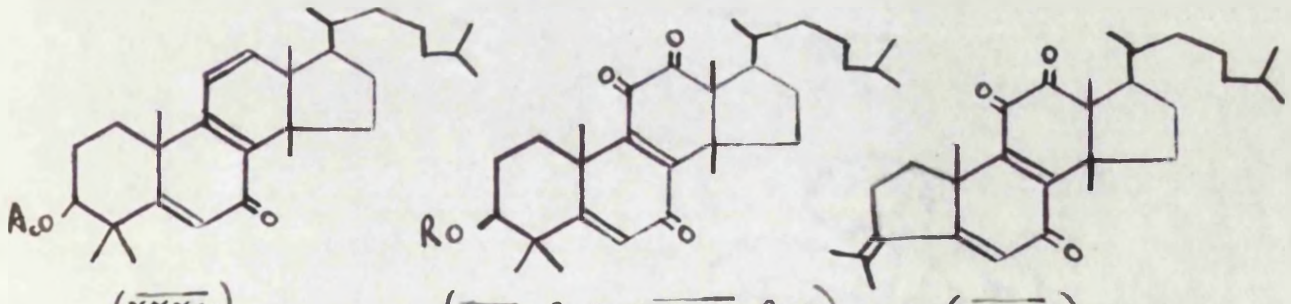
The earliest systematic oxidations of lanosterol were carried out by the Ruzicka school (27). Chromic acid oxidation of lanost-8-enyl acetate gave two products, 7:11-dioxolanost-8-enyl acetate (XXVIII) and 7-oxolanost--8-enyl acetate (XXIX). Later workers have found that more vigorous conditions give only the diketo-compound (XXVIII)(32-35) and that (XXIX) may readily be oxidised to (XXVIII) by chromic acid (32). 7:11-Dioxolanost-8--enyl acetate is typical of the transoid 'dione-enes' obtained by oxidation of the tetrasubstituted double bond present in most of the tetracyclic triterpenes. It has also been prepared by chromic acid oxidation of dihydroagnosteryl acetate (32) and by treatment of lanost-8-enyl acetate with hydrogen peroxide (28) or with ozone (36).

7:11-Dioxolanost-5:8-dienyl acetate (XXX) is formed by selenium dioxide oxidation of (XXVIII) (23,32).



(XXVIII) (XXIX) (XXX)

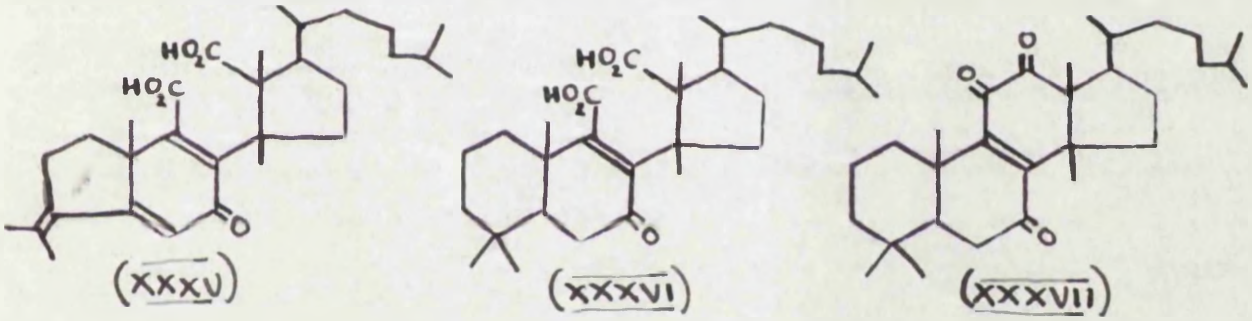
By a further, more drastic, selenium dioxide oxidation of (XXX) Rusicka (23) obtained 7:11:12-trioxolanost-5:8--dienyl acetate (XXXI). (XXXI) has also been prepared by chromic acid oxidation of 7-oxolanost-5:8:11-trienyl acetate (XXXII) (32). On treatment with phosphorus pentachloride, 7:11:12-trioxolanost-5:8-dienol (XXXIII) underwent a standard retropinacolinic change in ring A to give 7:11:12-trioxoisolanost-3:5:8-triene (XXXIV)(29).



(XXXII) (XXXI, R=Ac; XXXIII, R=H) (XXXIV)

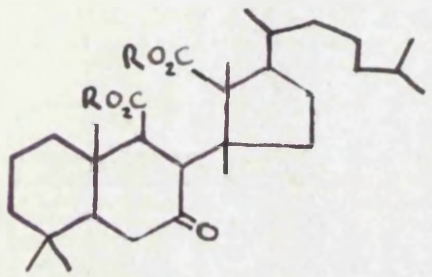
In this way Barton extended the conjugated system to include ring A and so related the position of the hydroxyl group in lanosterol to that of the inert double bond. Oxidation of the triene-trione (XXXIV) with alkaline hydrogen peroxide (29) opened ring C to give a keto-dicarboxylic acid (XXXV).

Jeger (37) has been able, by pyrolysis, to cleave a derivative of the related dicarboxylic acid (XXXVI) into two fragments.

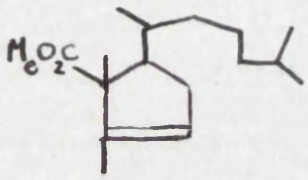


(XXXVI) was prepared by Jeger (37) from (XXXVII) (3). Reduction of (XXXVI) with zinc and acetic acid gave the keto-dicarboxylic acid (XXXVIII). The keto-dimethyl ester (XXXIX) was unchanged after alcoholic alkali saponification followed by remethylation. Thus in (XXXIX) the atoms $C_{(8)}$ and $C_{(9)}$ are in the thermodynamically most stable configurations. The keto-dimethyl ester (XXXIX) was pyrolysed in high vacuum at 350-360° and gave an enol--lactone, by an internal condensation, and, by a splitting

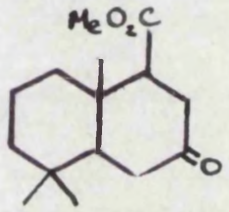
of the molecule, the unsaturated ester (XL) and the ketonic ester (XLI). By Clemmensen reduction of (XLI) the ester (XLII) was prepared. This ester was also derived by an unambiguous route from the diterpene, manool (XLIII).



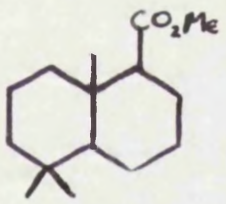
(XXXVIII, R=H; XXXIX, R=Me)



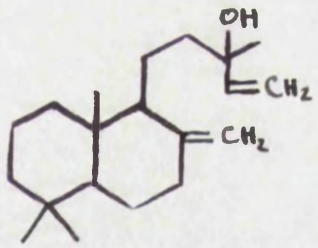
(XL)



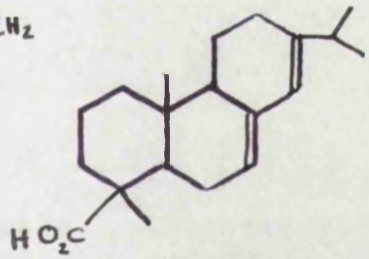
(XLI)



(XLII)



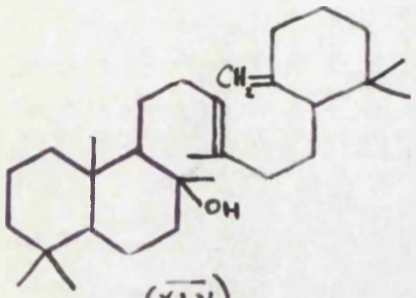
(XLIII)



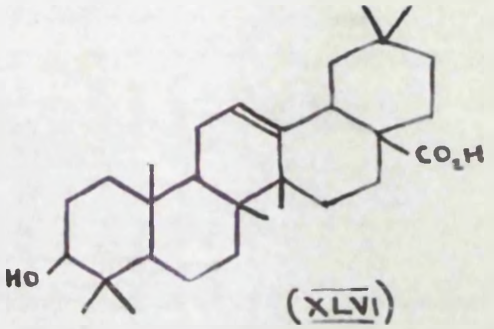
(XLIV)

Since the preparation of (XXXVII) from lanostadienol had involved no possibility of inversion at $C_{(5)}$ or $C_{(10)}$ and since manool had previously been related to abietic acid (XLIV) (38), ambrein (XLV)(39) and oleanolic acid (XLVI)(40) the above work showed that lanostadienol

has a structure and stereochemistry in rings A and B in common with a very large number of natural terpenes.



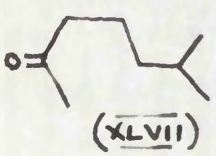
(XLV)



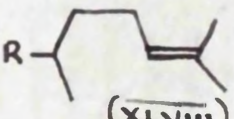
(XLVI)

Oxidations in the Lanosterol Side Chain.

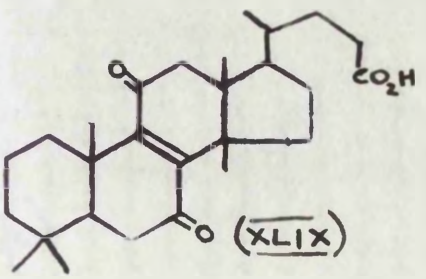
In the earliest oxidations of lanost-8-enyl acetate it was noted (41,42) that a sweet smelling, steam volatile substance was produced. Recently Barton, McGhie and co-workers (43) repeated this work on a large scale and were able, by careful chromatography of the 2:4-dinitrophenylhydrazone of the steam volatile portion, to show the presence of acetone and 6-methylheptan-2-one (XLVII). This result, together with what was known of the position of the side chain double bond, was taken as proof of the existence of the side chain as shown in formula (XLVIII) in lanosterol.



(XLVII)



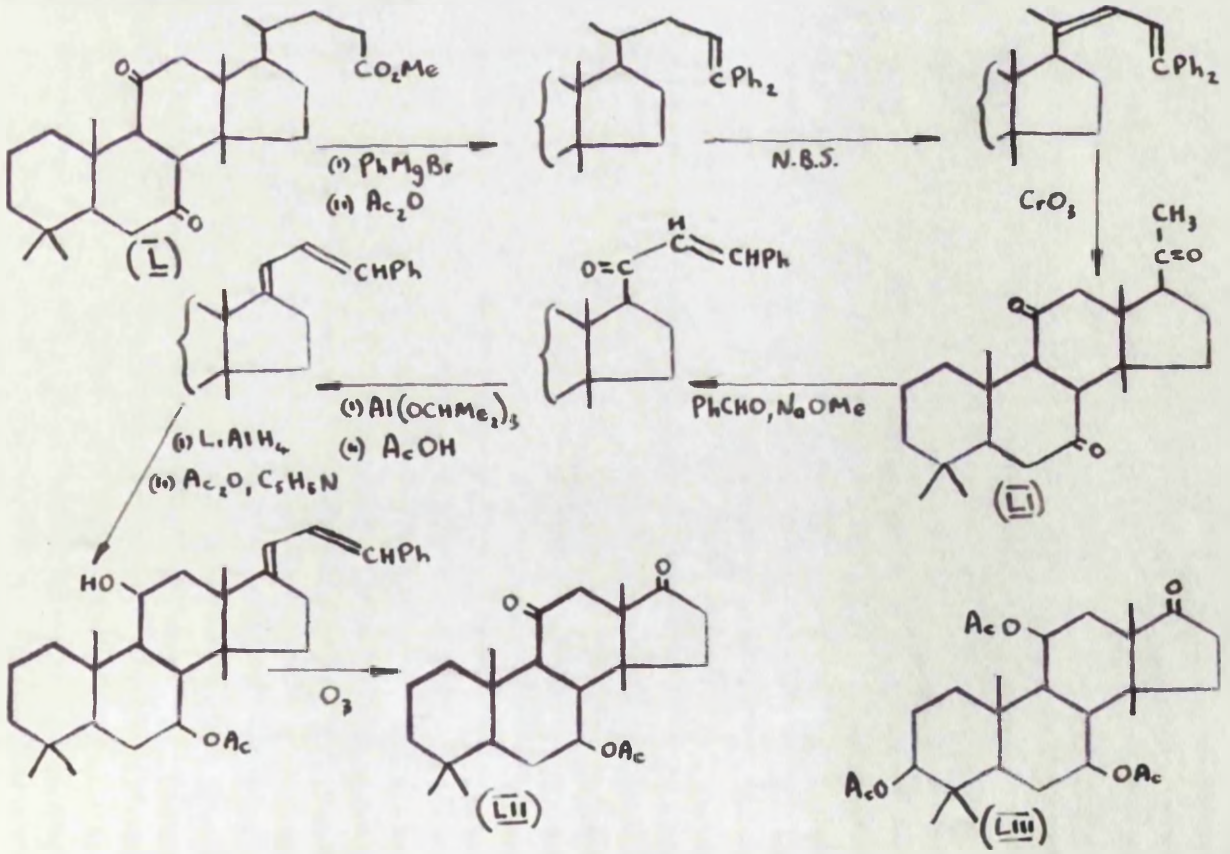
(XLVIII)



(XLIX)

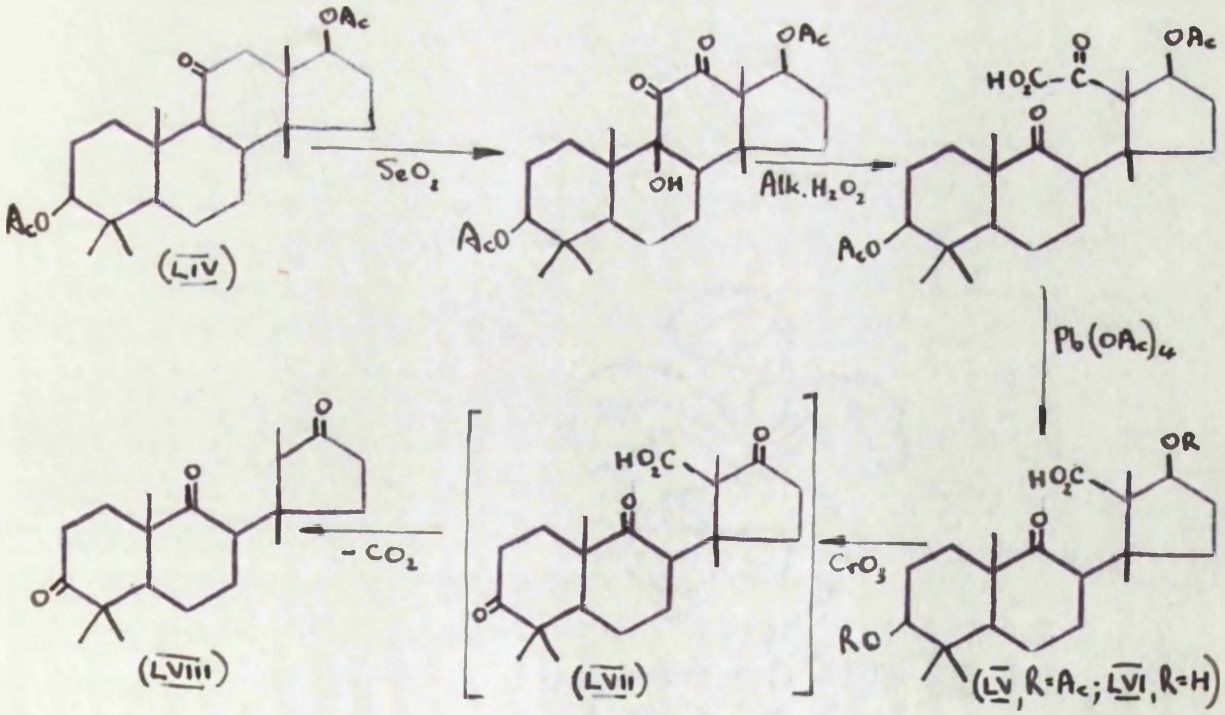
Independently the Ruzicka school arrived at the same conclusion by stepwise degradation of the side chain. Oxidation of lanost-8:24-diene with chromic acid gave trisnorlanost-7:11-dioxo-8-enoic acid (XLIX)(44). Treatment of the ester with zinc and acetic acid gave the diketo-ester (L) which was converted by the steps shown to the triketone (LI)(44).

By the further series of reactions shown the diketo-acetate (LII) was obtained from (LI). The infra-red spectrum of (LII) shows that it has a carbonyl group in a five-membered ring.



Barton (46) deduced from the integrated absorption intensity at 1412 cm.$^{-1}$ of a similar degradation product (LIII) that there is only one methylene group adjacent to the carbonyl group. This eliminated $C_{(16)}$ as the location of the carbonyl group in (LIII) and as the point of attachment of the side chain in lanosterol.

Ruzicka (47) finally decided between $C_{(15)}$ and $C_{(17)}$ by the following series of reactions. The degradation product (LIV) was converted to (LV) as outlined. When the dihydroxy-keto-acid (LVI) was oxidised under mild conditions, spontaneous decarboxylation took place giving the triketone (LVIII). This proves that (LVI) is a



β-hydroxy-acid giving rise to an intermediate β-keto-acid (LVII). These facts can only be accommodated by situating the hydroxyl in (LVI) and hence the side chain in lanosterol at $C_{(17)}$.

Stereochemistry of Lanosterol.

In 1952 (48), Curtis, Fridrichsons and Mathieson were able, from X-ray diffraction studies on lanosterol iodoacetate, to show that the side chain is fused to $C_{(17)}$, and to decide the configuration at each centre of asymmetry. Barton in 1953 (49) on the basis of conformational and generalised molecular-rotation arguments arrived at the same conclusions by chemical means and it is these arguments that will be outlined in this section.

Barton begins by accepting the molecular-rotation arguments of Klyne (50) that rings A and B are trans-fused as in the other triterpenoids that have been investigated (51). The work of Jeger (37) described above, in which the fragment of the lanosterol molecule corresponding to rings A and B is derived from manool, relates lanosterol to oleanolic acid. Oleanolic acid has been shown (52) to have rings A and B trans-fused.

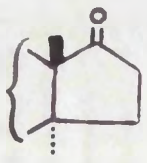
On the basis of its stability towards attempted epimerisation (53) and its regeneration on reduction of

suitable ketones with sodium and alcohol (42) the hydroxyl group at $C_{(3)}$ is taken to have the 3β-configuration. This is confirmed (54,51) by the well-known retropinacoline rearrangement of ring A observed on treatment with phosphorus pentachloride (55,56).

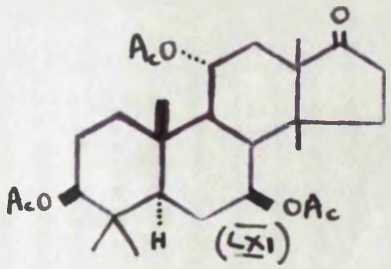
In a previous paper (29) Barton, in order to explain the differing degrees of steric hindrance of the keto-groups in lanost-7:11-dioxo-3-ol, argued that the $C_{(13)}$ methyl group is on the same side of the molecule as the $C_{(10)}$ methyl group and thus the $C_{(11)}$ carbonyl is hindered by two polar type $C-CH_3$ bonds.

The stereochemistry of the $C_{(14)}$ methyl group is established by the generalised molecular rotation method. The possibilities (LIX) and (LX) are considered for ring D of 3β:7β:11α-triacetoxylanan-17-one (LXI)(57). The molecular rotation difference on Wolff-Kishner reduction of the 17-keto-group was +110°. The approximate molecular rotation contributions to be expected for structures (LIX) and (LX) are (50) +500° and +250° respectively. Thus structure (LX) is favoured.

(LIX)

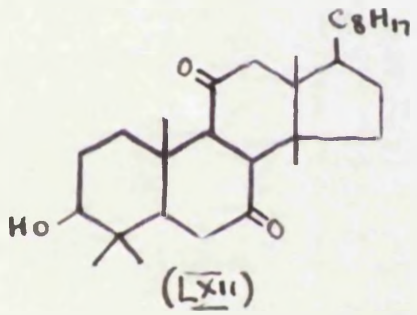


(LX)

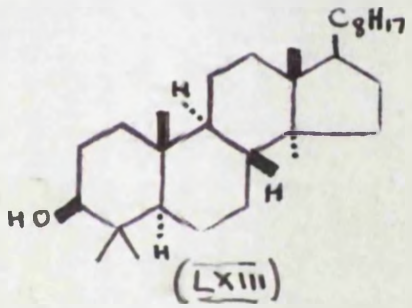


(LXI)

Compounds having rings B and C saturated are prepared *via* intermediates having 7:11-diketo-groupings. Lanost--7:11-dioxo-3-ol (LXII) is shown to be stable to vigorous alkaline treatment. Hence $C_{(8)}$ and $C_{(9)}$ are in the most stable configuration. On the basis of conformational arguments previously advanced by Barton (54) the configurations at $C_{(8)}$ and $C_{(9)}$ are *trans* relative to each other and *anti* relative to $C_{(14)}$ and $C_{(10)}$. The stereochemical conclusions so far are summarised for lanan-2-ol in (LXIII).



(LXII)



(LXIII)

The stereochemistry of the side chain is decided by considering the molecular rotation differences between compounds having the *iso*octyl side chain and those in which the side chain is replaced by -COMe. It is found that the values correspond closely to those for the 17β configuration in the steroid series and, therefore, the lanosterol side chain is fused 17β.

THEORETICAL

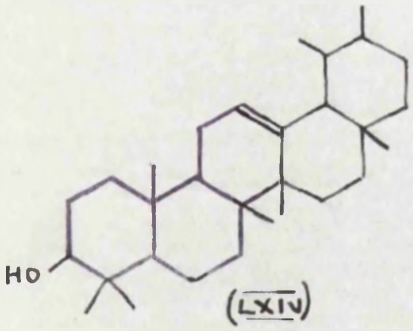
SECTION I

The triterpenoid components of the non-saponifiable fraction from the seed fat of Strychnos nuxvomica L., were identified as α-amyrin and cycloartenol. cycloArtenol was shown to be cyclolanost-24-enol containing a cyclopropane bridge extending from $C_{(9)}$ to $C_{(10)}$.

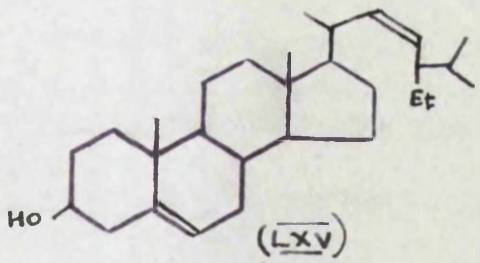
The seeds of Strychnos nuxvomica L. have had a commercial importance for over a hundred years because of the presence of the alkaloid poisons strychnine and brucine which were discovered by Pelletier and Caventou early in the last century. S.nuxvomica also contains from one to two per cent of a fat which was investigated as early as 1815 by Meyer (53) and found to consist mainly of the glycerides of capric, caprylic, caproic, butyric and palmitic acids. More recently (1912-1915) Heiduschka and Wallenreuter (59,60) made a detailed examination of the non-saponifiable fraction which they found to be about 20 per cent of the total fat. By fractional crystallisation from the acetic anhydride solution in which this material was acetylated, three crystalline acetates were separated and characterised as the alcohols. One of these alcohols, m.p.158°, was described as a phytosterol, analysis of which corresponded to the formula $C_{27}H_{46}O,H_2O$; a second alcohol, m.p.115° (99° solvated; acetate, m.p. 123-124°) was formulated as $C_{32}H_{54}O$ and a third, m.p.136° (acetate m.p.221°) as $C_{35}H_{58-60}O$. Several esters of the alcohol, m.p.136°, were prepared and it was observed that oxidation of the acetate, m.p.221°, with chromic acid gave a product resembling ''oxy-amyrin acetate''.

Through the courtesy of the Directors of Messrs. T. & H. Smith, Limited, Edinburgh, a quantity of S.nuxvomica seed fat was made available for further examination. In the commercial extraction of strychnine and brucine the seeds are crushed, extracted with boiling benzene and the alkaloids isolated from the benzene by extraction with hydrochloric acid solution. The brown semi-solid fat obtained by evaporation of the benzene was hydrolysed in 5 per cent methanolic sodium hydroxide by refluxing for 4 hours. The reaction mixture was concentrated, added to a large volume of water and the resulting suspension extracted with ether. In this way, yields of 12 to 15 per cent of non-saponifiable matter have been obtained as orange waxy solid. This was acetylated, dissolved in light petroleum and chromatographed on activated alumina to give four well-defined fractions. The first, a fragrant colourless oil has not been examined in detail. The second and largest fraction was crystallised from chloroform--methanol as plates, m.p.223-225°, and characterised by hydrolysis to the alcohol which crystallised from aqueous methanol as needles, m.p.135°. These compounds which gave a pink Liebermann-Burchard reaction and a strong

yellow colour with tetranitromethane were shown by mixed melting point determination, optical rotation and analysis to be α-amyrin (LXIV) and α-amyrin acetate; they correspond to the alcohol, m.p.136°, and acetate, m.p.221°, described by Heiduschka and Wallenreuter (59,60). M.B.E. Fayez (61) purified the final fraction to obtain an acetate, m.p.142.5-143°, hydrolysis of which gave an alcohol, m.p.165.5-167.5°. By comparison with an authentic specimen this alcohol was shown to be stigmasterol (LXV). It is likely that the phytosterol, m.p. 153°, giving a green Liebermann-Burchard reaction, described by Heiduschka and Wallenreuter (59,60) was an impure sample of stigmasterol.



(LXIV)



(LXV)

The third fraction was crystallised several times from chloroform-methanol to give plates, m.p.122-124°, in quantity corresponding to 10 per cent of the original acetylated non-saponifiable matter. Analyses of this

compound corresponded to a formula, $C_{32}H_{52}O_2$. The Liebermann-Burchard test gave an initial yellow which developed to a blood red colour having a green fluorescence. Unsaturation was evidenced by a strong tetranitromethane colour and this was confirmed by the ultraviolet absorption spectrum which showed $\lambda_{max.}$ 2090 Å (ϵ max. 1480). The compound $C_{32}H_{52}O_2$, m.p.122-124°), was shown to be an acetate by hydrolysis to the alcohol, m.p.99° (raised to 115° after rigorous drying) which analysed for formula $C_{30}H_{50}O$. This alcohol corresponds to the compound having identical melting points which was described by Heiduschka and Wallenreuter and assigned the formula $C_{32}H_{54}O$. The alcohol readily formed a benzoate, $C_{37}H_{54}O_2$, m.p.130°, and was shown to have a secondary hydroxyl group by the formation of a ketone, $C_{30}H_{48}O$, m.p.105-106°, on mild treatment with chromic acid.

The acetate, $C_{32}H_{52}O_2$, m.p.122-124°, was readily hydrogenated at room temperature in presence of platinum catalyst to give a dihydro-acetate, $C_{32}H_{54}O_2$, m.p.130--132°. This acetate was further characterised by preparation of an alcohol, $C_{30}H_{52}O$, m.p.99° (raised to 106° after vigorous drying) and thence a ketone, $C_{30}H_{50}O$, m.p.110°. All of these dihydro compounds showed a pale

yellow colour with tetranitromethane in chloroform but showed no selective absorption in the ultra-violet to indicate double bond unsaturation.

The acetate, $C_{32}H_{52}O_2$, m.p.122-124°, was treated with a large excess of perbenzoic acid in chloroform at 0°. After 16 hours, titration of a sample indicated reaction with 1 molecular equivalent of perbenzoic acid. No further reaction had taken place after a total time of 64 hours. When a sample of dihydro-acetate was treated in exactly the same manner no reaction whatever was observed after 64 hours. From a subsequent experiment in which acetate, m.p.122-124°, was treated with a molar quantity of perbenzoic acid there was isolated an acetate epoxide, $C_{32}H_{52}O_3$, m.p.144°, which showed a pale yellow colour with tetranitromethane but no selective absorption in the ultra-violet.

Further proof of the presence of a reactive double bond was obtained by titration of a cold acetic acid solution of acetate, $C_{32}H_{52}O_2$, m.p.122-124°, with a solution of 1 molecular equivalent of bromine in acetic acid. An acetate dibromide, $C_{32}H_{52}O_2Br_2$, m.p.163-167°, was isolated in good yield. As for the acetate epoxide and the dihydro series, this compound gave a pale yellow

colour with tetranitromethane and showed no selective absorption in the ultra-violet.

The nature of the reactive double bond was demonstrated by D.S. Irvine (61) by ozonolysis of the acetate, m.p.122-124°, to give acetone. In a subsequent experiment, the major fragment was also isolated as a trisnor-acid acetate, $C_{29}H_{46}O_4$, m.p.221.5-223°, characterised as the methyl ester. This work indicated that the reactive double bond was present as an isopropylidene group possibly terminating a side chain as in lanosterol.

Some conclusions as to structure were drawn from the results so far described. The analysis results for all the products fitted best a formula $C_{30}H_{50}O$ for the parent alcohol. The low-melting nature of all these compounds appeared to preclude a pentacyclic structure of amyrin or lupeol type and the ozonolysis results supported a tetracyclic structure having a side chain, as in lanosterol and euphol. Also the Liebermann-Burchard test was quite different from those of the pentacyclic triterpenes or the steroids. A tetracyclic structure required that the alcohol, $C_{30}H_{50}O$, be doubly unsaturated. However, the only evidence of unsaturation besides that of the reactive isopropylidene group was the persistent pale yellow

tetranitromethane colour shown by all compounds in which this group had been saturated. The dihydro-acetate did not react with perbenzoic acid and was recovered unchanged after prolonged treatment with chromic acid (in quantity corresponding to 9 equivalents of oxygen) at 70°. This stability to vigorous oxidising conditions and the fact that no selective absorption was shown between 1950 and 2200 Å (24,62) suggested that nuclear unsaturation might take the form of a cyclopropane or cyclobutane bridge. In support of this it was found that treatment of dihydro-acetate in chloroform with dry hydrogen chloride gave a product, m.p.137-157°, showing a strong yellow colour with tetranitromethane in chloroform and an ultra-violet absorption spectrum having λ max. 2080 Å (ϵ max. 3180).

The probability of a cyclopropane ring prompted comparison of the Strychnos nuxvomica compound with the only known triterpenoid containing such a grouping which Barton (63) had recently isolated from the latex of the fruit of Artocarpus integrifolia and named cycloartenol. A comparison of the physical constants of the S.nuxvomica alcohol and its derivatives with those of the corresponding derivatives described by Barton suggested identity; the identities of the alcohol and its acetate have been

confirmed by mixed melting point determinations kindly made by Professor D.H.R. Barton. The comparison of constants is set out in Table A.

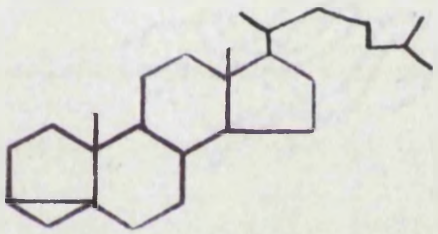
TABLE A

	From S. nuxvomica		From A. integrifolia		
	M.p.	$[\alpha]_D$	M.p.	$[\alpha]_D$	Mixed m.p.
cycloArtenol	115° (99°solvated)	+54°	85-92°	+48°	112-113.5° (sinters 110°)
Acetate	122-124	+59.5	122.5-123.5	+58	121.5-122.5
Benzoate	130	+76	129-130	+65	-
cycloArtenone	105-106	+22	109	+24	-
cycloArtanol	99	+50	99-101	+45	-
Acetate	130-132	+59	132-133	+57	-

cycloArtenol has since been isolated as a minor constituent of the latex of Euphorbia balsimifera by Chapon and David (64). These authors also suggested that cycloartenol might be identical with handianol, isolated from Euphorbia handiensis by Gonzalez and co-workers (65,66). In a very recent publication it has been reported (67) that these compounds are indeed identical.

From Artocarpus integrifolia Barton isolated mainly

cycloartenone together with small quantities of cycloartenol and butyrospermol; his conclusions about the structure of cycloartenol were essentially the same as those given above. The pale yellow tetranitromethane colours given by cycloartanol and its derivatives were noted and it was further observed that carefully purified i-cholestane (LXVI) gives a similar test and also shows no selective absorption in the ultra-violet in the 1960-2100 Å region.



(LXVI)

cycloArtanyl acetate was shown to be resistant to oxidation by chromic acid, selenium dioxide, perbenzoic acid and even by hot peracetic acid under conditions adequate for attack on the very resistant double bond of α-amyrin benzoate (68). Additional proof of the presence of a cyclopropane ring was found in the infra-red absorption spectra of cycloartenone and cycloartanyl acetate which have bands near 1000 cm.$^{-1}$ though partly masked by

neighbouring absorption bands of greater intensity. i-Cholestane showed intense absorption at 1010 cm.$^{-1}$ and Derfer, Pickett and Boord (69) have shown by study of a number of alkyl-substituted cyclopropane derivatives that the ring is characterised by an intense band at 1020-1000 cm.$^{-1}$.

Barton by treatment of cycloartanyl benzoate with dry hydrogen chloride in chloroform obtained an apparently pure isomer, artenyl benzoate which melted sharply, m.p.197-198°. Hydrolysis of this gave artenol which also melted sharply, m.p.152-154°. However, on acetylation of the alcohol the product only by repeated recrystallisation gave an apparently pure artenyl acetate, m.p.165-167°, having λ max. 1995 Å (ϵ max. 3600). Also, when cycloartanyl acetate was treated with hydrogen chloride some difficulty was found in purifying the product. Though these products were quoted as homogeneous it was allowed that artenol and its benzoate might be contaminated by double bond isomers not readily separable by crystallisation.

As mentioned above, treatment of cycloartanyl acetate (obtained via S.nuxvomica) in chloroform with dry hydrogen chloride gave a product, m.p.137-157° showing

ethylenic unsaturation. Attempts were made to resolve what was thought to be a mixed crystal composed of double-bond isomers. Repeated recrystallisation of the acetate mixture, m.p.137-157°, was accompanied by a gradual and continuous increase in melting point and great loss of material. The purest material obtained in this way had m.p.157-159°. Careful chromatography of the product, m.p.137-157° showed no separation. Hydrolysis of the original acetate mixture gave an alcohol mixture which melted sharply, m.p.152-154°, as observed by Barton for artenol, and which on reacetylation gave an acetate, m.p.137-160°. The derived ketone also melted sharply, m.p.121-123°, showed no change in melting point on repeated recrystallisation and no separation of isomers on careful chromatography.

With the object of obtaining a homogeneous product, the rearrangement of cycloartanyl acetate was attempted using acidic reagents other than hydrogen chloride. The acetate was treated in a refluxing ethanol, sulphuric acid, water mixture, was kept at room temperature for 7 days in a dioxan, sulphuric acid, water solution, and was kept at room temperature for 6 days with boron trifluoride etherate in benzene. Starting material only was recovered in

high yield from these experiments.

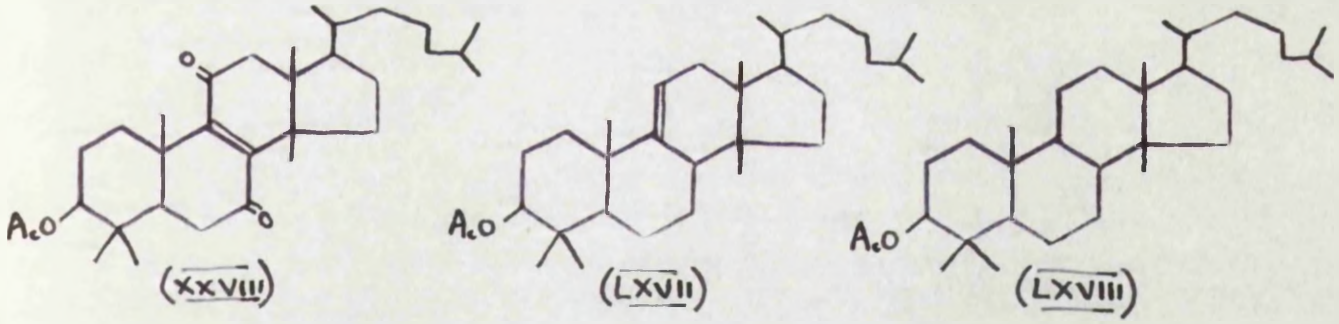
A homogeneous product was finally obtained by a procedure analogous to that used by Marker, Wittle and Mixon (42) to isolate lanost-7-enyl acetate from the rearrangement product of lanost-8-enyl acetate. The acetate mixture, m.p.137-157°, was treated under mild conditions with chromic acid in acetic acid; the component which largely escaped oxidation was readily separated in about 50 per cent yield by chromatography and crystallised as hexagonal plates, m.p.170-172°. It showed a strong yellow tetranitromethane colour and an ultra-violet absorption spectrum having λ max. 2060 Å (ϵ max. 4300). From this chromatogram there were isolated small fractions which although they could not adequately be purified showed ultra-violet absorption at 2700 Å characteristic of a fully transoid 1:4-dione-ene system. The isolation of the pure compound from another source is described below. From the last eluted fractions of this chromatogram was isolated a small amount of an acetate, $C_{33}H_{52}O_3$, m.p.184-185°, which gave no tetranitromethane colour and showed an ultra-violet light absorption spectrum having λ max. 2420 Å (ϵ max. 9,800) characteristic of an $\alpha\beta$-unsaturated ketone having

ββ-disubstitution. This compound was shown to have originated from oxidation of a small amount of the unsaturated acetate, m.p.170-172°, by oxidising the pure acetate under more vigorous conditions with chromic acid, when the αβ-unsaturated ketone, m.p.184-185°, was obtained in good yield together with some unchanged starting material.

D.S.Irvine (61) resolved the acetate-mixture, m.p. 137-157°, by a modification of the above procedure. The material in acetic acid was shaken with hydrogen and platinum at 30° for 24 hours. The product which still showed a yellow tetranitromethane colour and a reduced absorption at 2070 Å (ϵ = 2600) was treated with chromic acid in acetic acid and then chromatographed. A saturated acetate, $C_{32}H_{56}O_2$, m.p.155-156° was obtained in 60% yield. Further elution gave a 10% yield of a yellow compound, m.p.155-156°, which gave no colour with tetranitromethane and had light absorption maximum at 2700 Å (ϵ = 7600). As mentioned above, this ultra-violet spectrum is characteristic of a fully transoid 1:4-dione--ene system as in 7:11-dioxolanost-8-enyl acetate (XXVIII).

Since, as shown in the historical section of this thesis, most of the known tetracyclic triterpenoids have

been related either to euphol or lanosterol (1) it was hoped that the above described compounds, formed by breaking the cyclopropane ring of cycloartanol, might establish such a relationship. A relationship with lanosterol was in fact shown. By a comparison of properties and physical constants it appeared that the unsaturated acetate, m.p.170-172°, might be identical with lanost-9(11)-enyl acetate (LXVII), the saturated acetate, m.p.155-156°, with lanostanyl acetate (LXVIII) and the acetate-ene-dione, m.p.155-158°, with 7:11-dioxo-lanost-8-enyl acetate (XXVIII).



(XXVIII) (LXVII) (LXVIII)

The comparison of constants is given in Table B below.

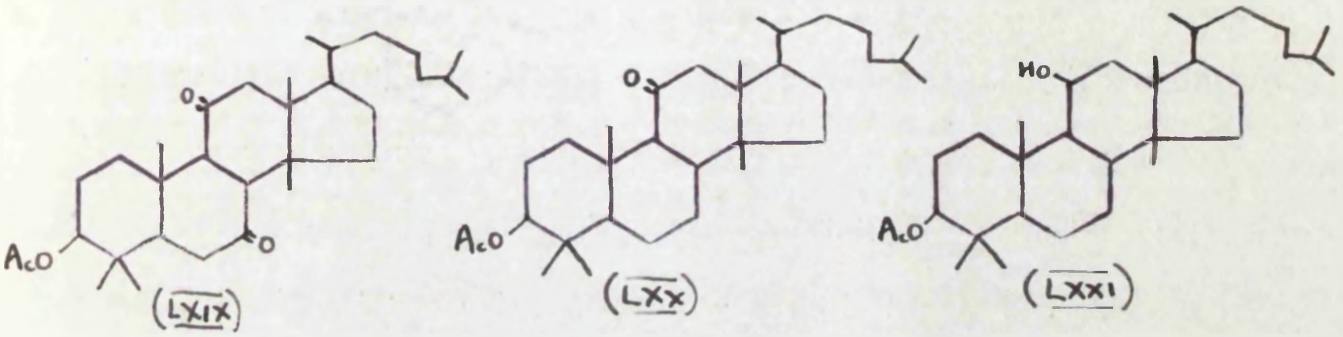
TABLE B

	M.p.	$[\alpha]_D$	λ max.	ϵ
Lanost-9(11)-enyl acetate				
From cycloartanyl acetate	170-172°	+85°	2060Å	4300
Ruzicka et al. (28)	170-171	+81		
McGhie et al. (20)	177-178	+83		
7:11-Dioxolanost-8-enyl acetate				
From cycloartanyl acetate	155-156	+91	2700	7600
Ruzicka et al. (10)	156-158	+90.5		
McGhie et al. (33)	158-159	+91.6	2750	8700
Lanostanyl acetate				
From cycloartanyl acetate	155-156	+41	-	-
Ruzicka et al. (28)	150-151	+41	-	-
McGhie et al. (70)	156-157	+45	-	-

In order to prove identity by direct comparison the compounds given above were prepared using as starting material a sample of ''isocholesteryl acetate'' kindly supplied by Dr. C.L. Hewitt of Organon Laboratories Limited. ''isoCholesterol'' is known (10) to be composed

of lanosterol, dihydrolanosterol, agnosterol, and dihydroagnosterol. Accordingly, hydrogenation of the acetate mixture with platinum in acetic acid gave a mixture of dihydrolanosteryl acetate and dihydroagnosteryl acetate. Since chromic acid oxidations of dihydrolanosteryl acetate (32-35) and of dihydroagnosteryl acetate (32) are known to give 7:11-dioxolanost-8-enyl acetate, the hydrogenated mixed acetates were oxidised by a procedure based on that used by Cavalla and McGhie (33) to oxidise lanost-8-enyl acetate. 7:11-Dioxolanost-8-enyl acetate (XXVIII) was obtained in good yield and shown to be identical with the product derived from cycloartanyl acetate. Treatment of (XXVIII) with zinc dust in acetic acid (35) gave 7:11-dioxolanostanyl acetate (LXIX) which on Wolff-Kishner reduction under Huang-Minlon conditions as described by McGhie, Pradhan and Cavalla (70) was converted to 11-oxolanostanyl acetate (LXX). 11-Hydroxylanost-3-yl acetate (LXXI) was prepared as described by Ruzicka and colleagues (28) by lithium aluminium hydride reduction of 11-oxolanostanyl acetate followed by acetylation. Lanost-9(11)-enyl acetate (LXVII) was obtained by treatment of (LXXI) with phosphorus oxychloride in pyridine under conditions more vigorous than those used

by Ruzicka (28), and was shown to be identical with the rearrangement product of cycloartanyl acetate.

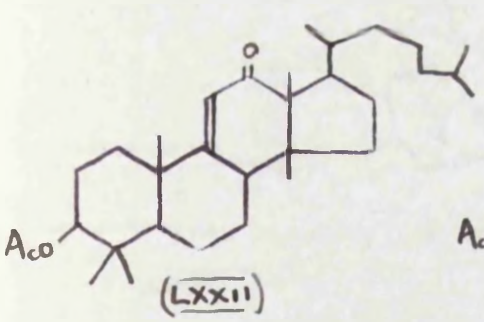


It is probable that the compounds described by Barton (63) as artenol, artenyl acetate and artanyl acetate were largely lanost-9(11)-enol, lanost-9(11)-enyl acetate and lanostanyl acetate respectively. Table C gives a comparison of the constants quoted (63) with those obtained in this work.

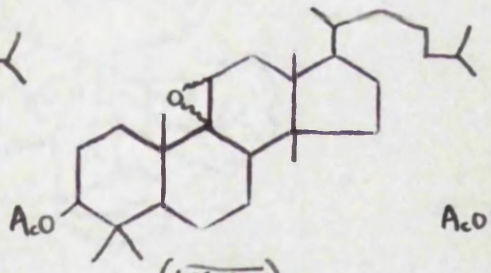
TABLE C

	M.p.	$[\alpha]_D$	λ max.	ϵ
Artenol (63)	152-154°	+57°		
Lanost-9(11)-enol(this work)	167	+76		
Artenyl acetate (63)	165-167	+73	1995Å	3600
Lanost-9(11)-enyl acetate (this work)	170-172	+85	2060	4300
Artanyl acetate (63)	148-149			
Lanostanyl acetate(this work)	156	+40.5		

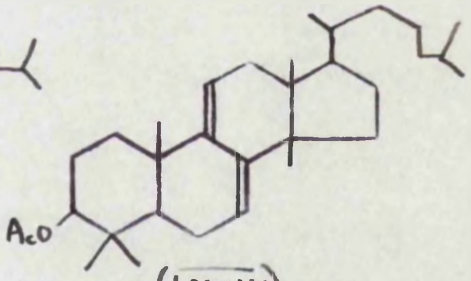
The identification of lanost-9(11)-enyl acetate as the major rearrangement product of cycloartanyl acetate means that the αβ-unsaturated ketone, m.p.134-135°, which it gave on chromic acid oxidation (see above) must be 12-oxolanost-9(11)-enyl acetate (LXXII). When 12--oxolanost-9(11)-enyl acetate was hydrogenated with platinum in acetic acid at 30° for 24 hours the product was lanostanyl acetate. By hydrogenation of (LXXII) with platinum in cold acetic acid for 6 hours the reaction was arrested after hydrogenolysis of the 12-keto-group and lanost-9(11)-enyl acetate was isolated.



(LXXII)



(LXXIII)



(LXXIV)

Oxidation of lanost-9(11)-enyl acetate with peracetic acid gave a saturated compound showing no colour with tetranitromethane and no selective absorption in the ultra-violet. This must be 9:11-epoxylanostanyl acetate (LXXIII) and is probably identical with the ''artenyl acetate oxide'' described by Barton (63) as the product of

similar treatment of artenyl acetate. Treatment of (LXXIII) with hydrogen bromide in acetic acid and chloroform gave in good yield a compound having a red-brown tetranitromethane colour and an ultra-violet absorption maximum at 2430 Å characteristic of a conjugated heteroannular diene system. This compound is probably identical with lanost-7:9(11)-dienyl acetate (LXXIV) which is isolated from ''isocholesteryl acetate'' (10). Table D shows a comparison of the constants obtained for this product with constants quoted (29,71) in the literature.

TABLE D

Lanost-7:9(11)-dienyl acetate

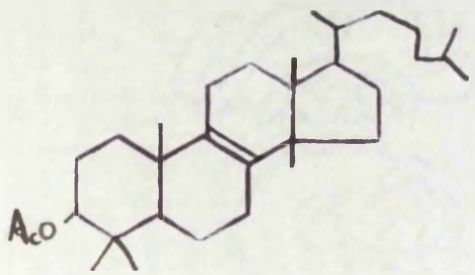
	This work	Barton et al.(29)	Ruzicka et al.(71)
M.p.	165-166°	165-166°	167-168°
$[\alpha]_D$	+87°	+87°	+87.8°
λ max.	2350Å(ϵmax.15,300)	2340Å(ϵmax.15,100)	
λ max.	2430Å(ϵmax.17,600)	2430Å(ϵmax.17,400)	
λ max.	2520Å(ϵmax.11,900)	2520Å(ϵmax.11,500)	

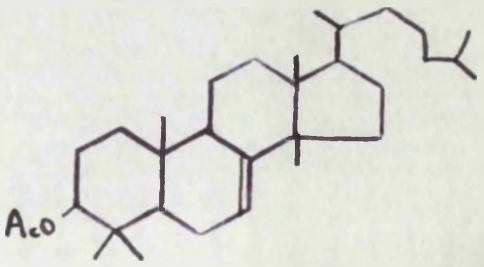
As already mentioned, from the rearrangement product of cycloartanyl acetate it was possible, by partial oxidation, to isolate lanost-9(11)-enyl acetate in about

50 per cent yield plus small amounts of 12-oxolanost--9(11)-enyl acetate or, by hydrogenation followed by oxidation, to isolate lanostanyl acetate in 50-60 per cent yield plus a 10 per cent yield of 7:11-dioxolanost--8-enyl acetate. Hence the major product of rearrangement is lanost-9(11)-enyl acetate.

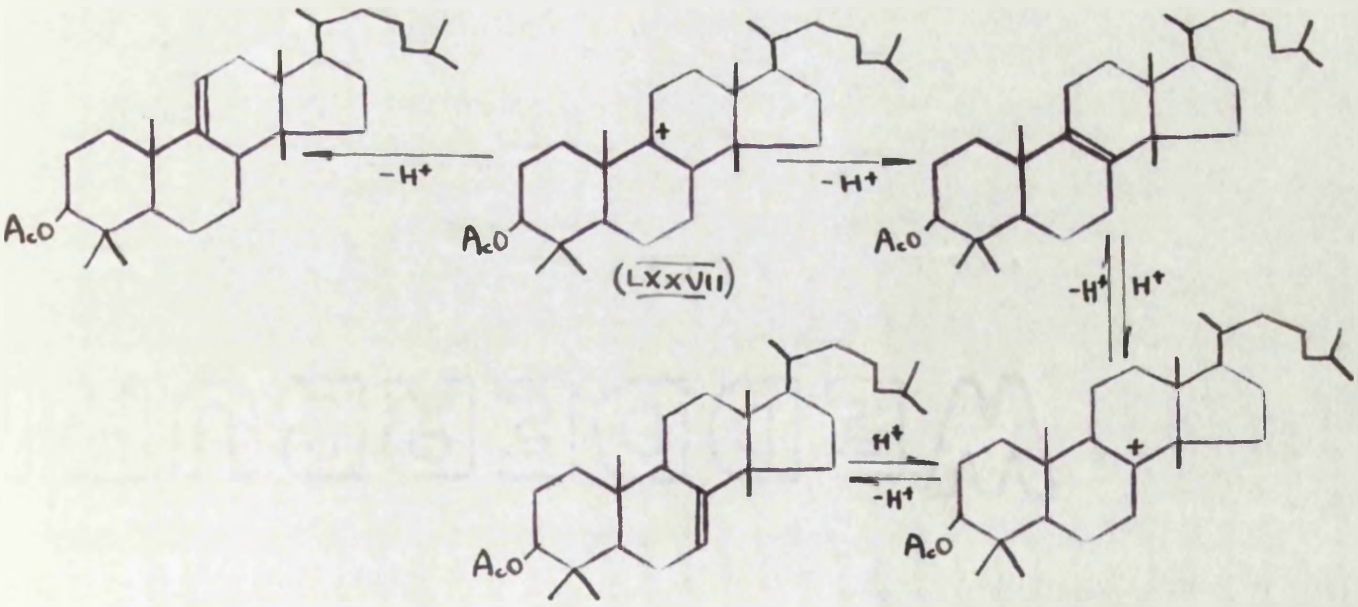
It has been shown (see above) that chromic acid oxidation of lanost-9(11)-enyl acetate gives 12-oxolanost--9(11)-enyl acetate alone, chromatography of the product revealing no trace of 7:11-dioxolanost-8-enyl acetate which is, however, known (27,72,23,73) to be readily formed on chromic acid oxidation of lanost-8-enyl acetate (LXXV) and lanost-7-enyl acetate (LXXVI). Further, lanost--9(11)-enyl acetate may be hydrogenated to lanostanyl acetate (23,70) whereas the Δ 8 and Δ 7 isomers are inert to hydrogenation (42,73). Thus it appeared that in the reaction described, which gave lanostanyl acetate as major and 7:11-dioxolanost-8-enyl acetate as minor product the former arose by hydrogenation of lanost-9(11)-enyl acetate and the latter by oxidation of unhydrogenated Δ 8 or Δ 7 isomers. However, the fact (42,73,29) that treatment of either lanost-8-enyl acetate or lanost-7-enyl acetate with hydrogen chloride in chloroform under the

same conditions as those used to rearrange cycloartanyl acetate gives an equilibrium mixture of the two isomers means that the rearrangement product of cycloartanyl acetate is probably a mixture of Δ9(11), Δ8 and Δ7 isomers. Though only 60-70 per cent of the rearrangement product has been accounted for, it seems unlikely that anything other than these three compounds is present. This belief was substantiated by crystallisation of a mixture of lanost-9(11)-enyl acetate (60%) and the equilibrium mixture of lanost-7- and -8-enyl acetate (40%) to give a mixed crystal closely resembling the acetate mixture, m.p.137-157°. Further, hydrolysis of the prepared acetate mixture gave an alcohol mixture which melted sharply, m.p.153-154°, as previously observed for the cycloartanyl acetate derived material. Mixed melting point determinations for the appropriate mixed crystals showed no depression.

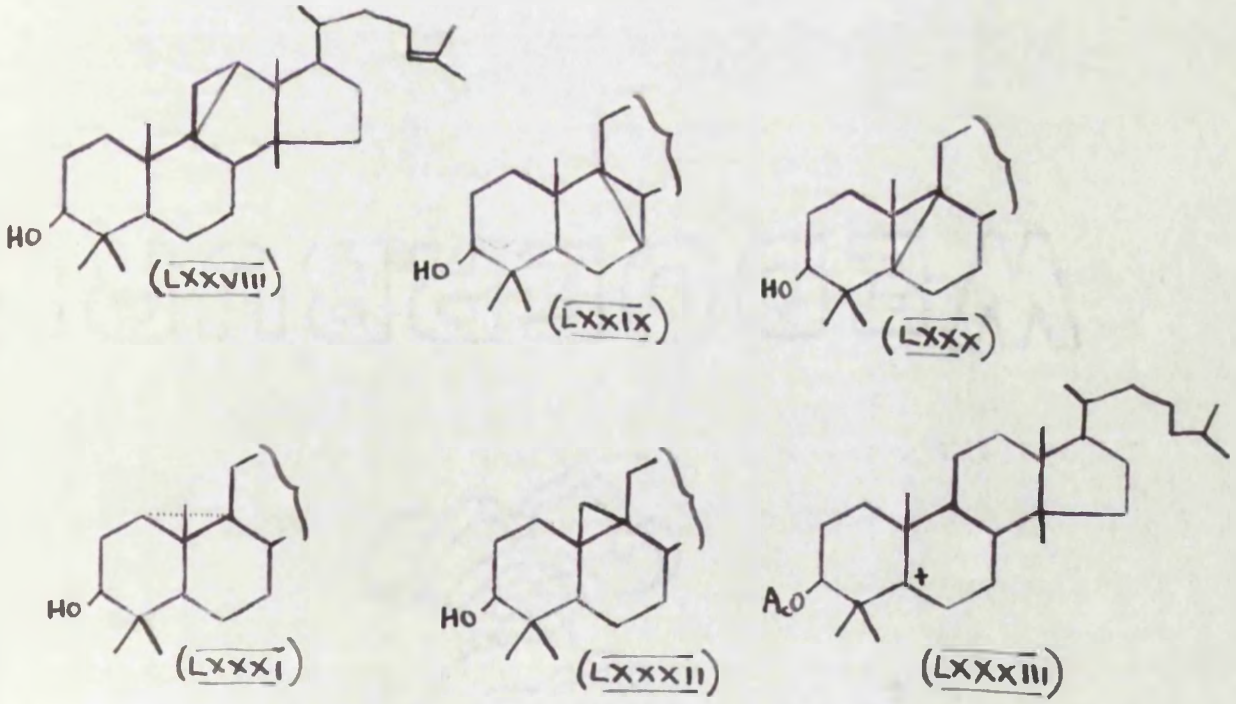




Treatment of lanost-9(11)-enyl acetate with hydrogen chloride in chloroform resulted in quantitative recovery of starting material; also in no case (42,73,29) has any trace of Δ 9(11) isomer been reported in a rearrangement product of Δ8 or Δ7 isomers. Thus it appears that lanost-9(11)-enyl acetate and the equilibrium mixture of lanost-7- and -8-enyl acetates are produced independently from the carbonium ion formed on breaking the cyclopropane ring and that no subsequent interconversions take place. The simplest interpretation of the above facts is that the carbonium intermediate (LXXVII) is the initial product of acid rearrangement and that this stabilises as shown in the scheme below.

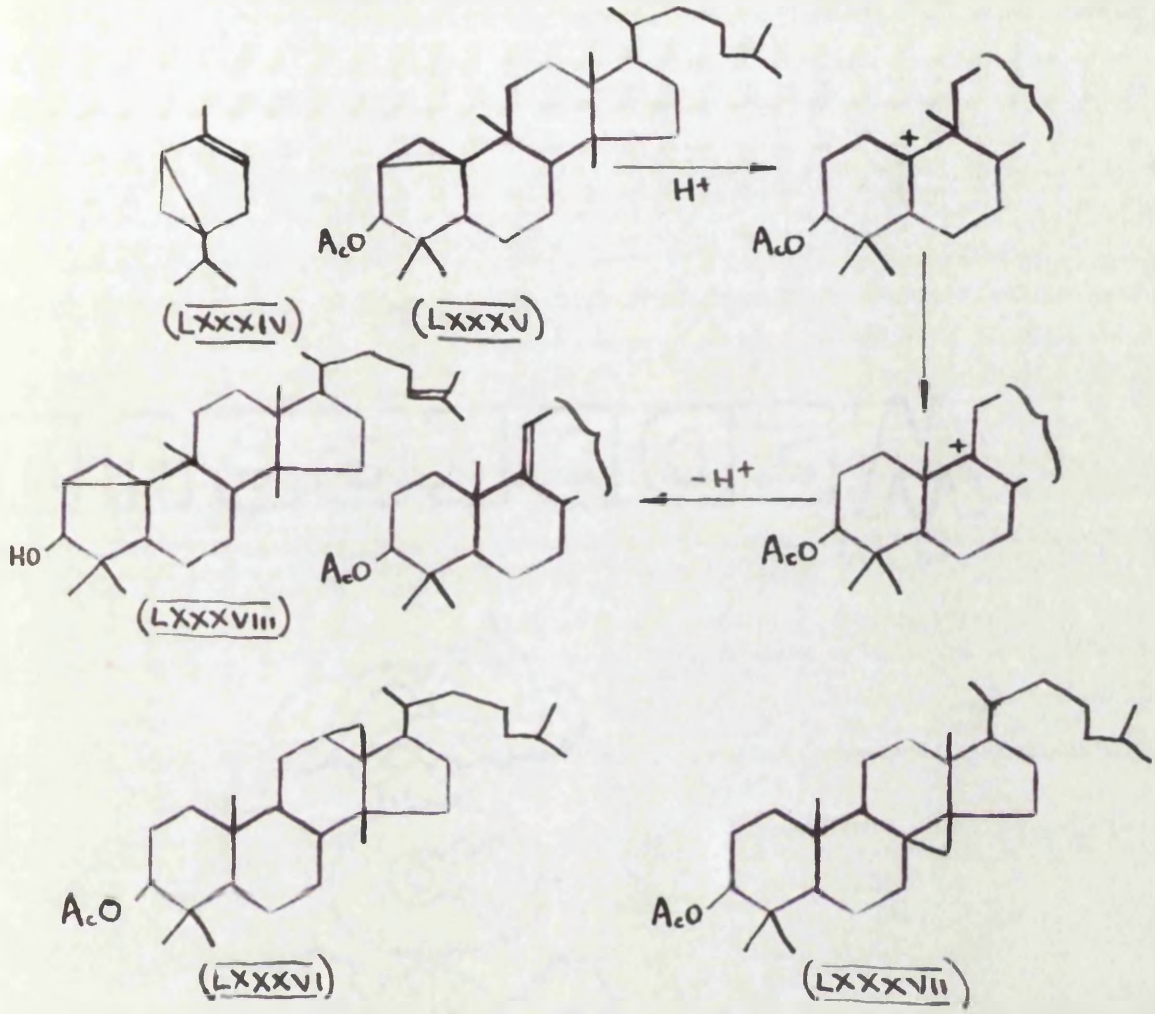


If modification of the lanostane carbon skeleton are for the moment excluded (further discussed below) the mechanism given demands that the cyclopropane bridge extend from $C_{(9)}$. Accordingly, on the evidence so far given, the structures (LXXVIII)-(LXXII) were all considered possible for cycloartenol. Structure (LXXX), however, appeared improbable since rearrangement products derived from carbonium ion (LXXXIII) would have been expected to occur in the mixed acetate product, m.p.137--157°.



Having located one end of the cyclopropane bridge at $C_{(9)}$ it was hoped that by rearrangement of cycloartanyl acetate using a cation other than H^+ the other end of the bridge might be ''marked''. D.S.Irvine (74) attempted bromination of cycloartanyl acetate using various conditions but found that by mild treatment with moderate excess of bromine only starting material could be recovered and more vigorous treatment yielded no crystalline product. The pyridine stabilised iodine salt of stearic acid described by Zingaro and colleagues (75) was thought to be a possible reagent for the introduction of iodine. This was prepared as described by these workers and cycloartanyl acetate treated in dry chloroform for 7 days at room temperature and under reflux for 2 hours. From both experiments starting material was recovered in high yield. From a study of the action of t-butyl hypochlorite on a series of phenols Ginsburg (76) suggested that with this reagent the chlorinating species may be a positive chloronium ion. With this in mind, cycloartanyl acetate was treated with t-butyl hypochlorite in dry carbon tetrachloride at room temperature and under reflux. However, as before, only starting material was recovered.

Work published by Cole (82) in September, 1953, made a valuable contribution to the evidence concerning the position of the cyclopropane ring. It was shown that the infra-red spectra of cycloartenol and cycloartanol have a band in the CH stretching region at 3042 cm.$^{-1}$ which is also observed for i-cholestane (LXVI) and α-thujene (LXXXIV) and which only appears when there is a CH_2 group included in the cyclopropane ring. This meant that the five possible structures (LXXVIII)--(LXXXII) were reduced to two, i.e. (LXXVIII)and (LXXXII). The five structures originally considered were arrived at while discounting simple modifications of the lanostane carbon skeleton. However, in the light of Cole's work, the structure (LXXXV) was considered for cycloartanyl acetate; its conversion into lanost-9(11)-enyl acetate, involving the usual mechanism for a methyl group migration, is shown below. Further formulae (LXXXVI) and (LXXXVII) which would agree with Cole's results were excluded by the knowledge that lanost-9(11)--enyl acetate may not rearrange to lanost-8-enyl acetate or vice versa.



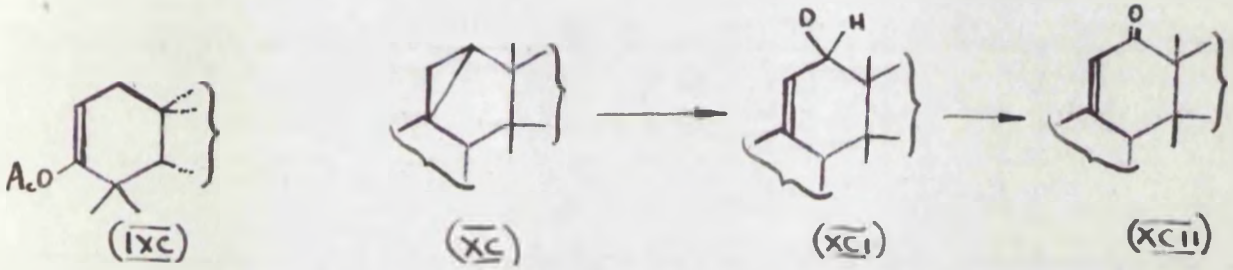
(LXXXIV) (LXXXV) (LXXXVI) (LXXXVII) (LXXXVIII)

Formula (LXXXVIII) for cycloartenol has been proved untenable by the fact that treatment of cycloartanone with isopropenyl acetate and a trace of sulphuric acid gave in good yield an enol acetate, $C_{33}H_{52}O_2$, which was readily hydrolysed to the parent ketone. This enol acetate must have the structure (IXC) in ring A which, therefore, cannot contain the bridge shown in (LXXXVIII).

Further, careful examination of the ultra-violet absorption spectrum of cycloartanone does not reveal the characteristics associated with a cyclopropane ring in conjugation with a carbonyl group (77).

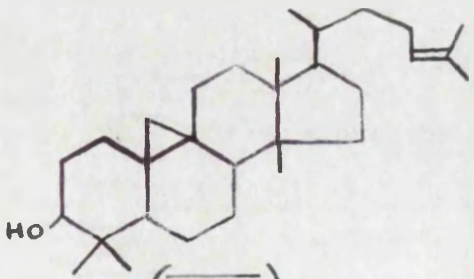
A method for the examination of formula (LXXVIII) was suggested by the work of Barton and de Mayo (78) on phyllanthol, a new triterpene which they have recently shown to have a cyclopropane ring included in the α-amyrin carbon skeleton. cycloArtanyl acetate was treated with deuterium chloride in pure chloroform in presence of a trace of deuterium oxide and, after the partial oxidation procedure adopted for the undeuterated compound, gave x-deuterolanost-9(11)-enyl acetate which showed the same physical constants and ultra-violet light absorption spectrum as lanost-9(11)-enyl acetate. Deuterium micro--analysis of this compound which was done by the kind co-operation of Dr. T.F. Gallagher and Dr. D.K.Fukushima (of the Sloan-Kettering Institute, New York) showed a content of 0.815 gram-atoms per gram-molecule. Oxidation of x-deuterolanost-9(11)-enyl acetate with chromic acid in acetic acid gave x-deutero-12-oxolanost-9(11)-enyl acetate which also had constants the same as those of the undeuterated compound; this had a deuterium content of 0.811 gram-atoms per gram-molecule. If cycloartenol had

formula (LXXVIII), treatment of cycloartanyl acetate (XC) with deuterium chloride would give 12-deuterolanost--9(11)-enyl acetate (XCI), oxidation of which would yield the undeuterated 12-oxolanost-9(11)-enyl acetate (XCII); cycloartenol cannot, therefore, have formula (LXXVIII).



(IXC) (XC) (XCI) (XCII)

Since all other reasonable alternatives have been eliminated, cycloartenol must have structure (LXXXII) and the deuterated compounds described must be 19-deuterolanost--9(11)-enyl acetate and 19-deutero-12-oxolanost-9(11)--enyl acetate.



(LXXXII)

Simultaneously with the publication of the deuterium chloride work described above, Barton reported (79) an infra-red examination of the product from deuterium chloride treatment of cycloartane. The infra-red spectrum of the deuterated mixture of lanost-9(11)-, -8- and -7-ene was examined in the C-H bending region (80,81) and showed, by comparison of the intensity of absorption at 1372 cm.$^{-1}$ with that given by lanost-9(11)-ene, that one of the methyl groups in the last compound was replaced in the deuterated material by a deuteromethyl group. The only one of the suggested structures for cycloartenol which can by fission of the cyclopropane ring give a deuteromethyl group is (LXXXII) which is, therefore, substantiated by positive evidence.

SECTION II

A new triterpenol, cyclolaudenol, and d-nonacosan-10-ol have been isolated from the non-saponifiable fraction of a chloroform extract of opium marc. cycloLaudenol has been shown to be 24-methylcyclolanost-25-enol containing a cyclopropane bridge extending from $C_{(9)}$ to $C_{(10)}$.

Opium has been continuously examined for alkaloids since the beginning of the eighteenth century and from it about thirty different bases have been isolated and identified. Although the technical process for the extraction of the alkaloids is still essentially that described by Grigny in 1833 (89; cf. 90,91) it has been made highly efficient. In this method the opium is stirred with water and the mixture treated with a hot concentrated solution of calcium chloride. The mixture is filtered, the press-cake thoroughly washed with water and the filtrate and washings combined and processed for alkaloids. The press-cake or opium marc, which in this dry state corresponds to one fifth of the weight of the original opium, contains the calcium salts of meconic, lactic and sulphuric acids. In addition it should contain any water insoluble non-basic components of opium.

Through the courtesy of the Directors of T. and H. Smith, Limited, Edinburgh, a sample of opium marc was made available for examination. Chloroform extracts of the marc, after acid washing to remove residual alkaloidal material (mainly narcotine), yielded a dark gum (18 per cent of total weight); by hydrolysis of this in ethanolic potassium hydroxide, a non-saponifiable fraction (24 per

cent by weight) was obtained. About 50 per cent of this non-saponifiable material separated as a partly crystalline solid from concentrated acetone solution, and chromatographic examination of this product led to the isolation of two compounds having melting points 81-82° and 123-125° respectively. Chromatography of the residual material revealed a considerable quantity of crystalline solid (probably triterpenoid in nature) which did not readily give any homogeneous product and was set aside for further examination. It has been found that the acetone crystallisation stage is essential, direct chromatography of the non-saponifiable fraction yielding only mixed crystalline materials.

The compound, m.p.81-82°, which was eluted first in the chromatogram was obtained in quantity corresponding to 15 per cent of the acetone crystallised material. It had empirical formula, $C_{29}H_{50}O$, showed no colour with tetranitromethane or in the Liebermann-Burchardt test, no selective absorption in the ultra-violet and no optical rotation in high concentration. Acetylation under normal conditions to give an acetate, $C_{31}H_{52}O_2$, m.p.44.5-45.5°, and mild oxidation to give a ketone, $C_{29}H_{48}O$, m.p.74.5--75.5°, showed the substance to be a secondary alcohol.

Reduction of the ketone under Wolff-Kishner conditions yielded a hydrocarbon, $C_{29}H_{60}$, m.p.63-64°. Inspection of the literature concerning the limited number of secondary, long chain compounds which have been isolated from natural sources suggests that the alcohol, $C_{29}H_{60}O$, is identical with d-nonacoson-10-ol. Chibnall and colleagues (33) isolated this compound from apple cuticle wax and established its structure by synthesis of the derived ketone, n-nonacoson-10-one. Chibnall found that no optical rotation could be detected for the alcohol itself but the derived hydrogen phthalate in concentrated solution (20% in chloroform) had a small but definite rotation, $[\alpha]_{5461}$ +0.62°. The hydrocarbon, $C_{29}H_{60}$, appears to be identical with n-nonacosane which was first prepared by Chibnall (34) by Clemmensen reduction of n-nonacosan-15-one. Chibnall in 1931 (33) suggested that the alcohol isolated by Kawamura (35) from the fruit of Ginkgo bilboa was d-10-nonacosanol; this was confirmed in 1934 (36). Table E gives a comparison of constants.

TABLE E

	Opium (this thesis)	Apple cuticle (83)	Ginkgo biloba (85)
d-10-Nonacosanol	81-82°	81.9-82.2°	82.5°
d-10-Nonacosanyl acetate	44.5-45.5	44.5-45	43-43.5
n-Nonacosan-10-one	74.5-75.5	74.7-74.9	74
n-Nonacosane	63-64	62.7-63	-

The second compound, m.p.123-125°, which was eluted after d-10-nonacosanol, crystallised from methanol as beautiful needles, in quantity corresponding to about 35 per cent of the solid chromatographed. For reasons that will become clear from the sequel this compound has been named cyclolaudenol. On the evidence of the analysis figures for cyclolaudenol and its derivatives, each of the formulae $C_{30}H_{50}O$, $C_{31}H_{52}O$ and $C_{32}H_{54}O$ could be considered for the alcohol. By degradative evidence it has finally been shown that cyclolaudenol has the formula $C_{31}H_{52}O$ and, therefore, throughout this discussion, formulae based on this will be quoted, though much of the work was done without certain knowledge of molecular weight. cyclolaudenol in the Liebermann-Burchardt test gave a final blood red colour and green fluorescence

exactly as for cycloartenol; with tetranitromethane in chloroform it gave a pale yellow colour. The latter test together with the ultra-violet light absorption maximum at 2050 Å (ϵ = 1145) indicated ethylenic unsaturation. The alcohol was characterised by preparation of cyclolaudenyl acetate, $C_{33}H_{54}O_2$, and cyclolaudenyl benzoate, $C_{38}H_{56}O_2$, both of which were readily formed by the usual methods. As for cyclolaudenol, both esters gave pale yellow tetranitromethane colours and the acetate had ultra-violet absorption maximum at 2060 Å (ϵ = 1500). The hydroxyl group was shown to be secondary by oxidation of the alcohol with chromic acid at room temperature to give cyclolaudenone, $C_{31}H_{50}O$.

cycloLaudenyl acetate on hydrogenation at room temperature in presence of platinum catalyst absorbed one molecular proportion of hydrogen in 30 minutes after which hydrogen uptake ceased; cyclolaudanyl acetate, $C_{33}H_{56}O_2$, was isolated and hydrolysed to cyclolaudenol, $C_{31}H_{54}O$. The readily hydrogenated ethylenic linkage was further characterised by treatment of cyclolaudenyl acetate with perbenzoic acid at 0°, titration of a sample withdrawn after 24 hours indicating reaction with one atom of oxygen. cycloLaudenyl acetate oxide, $C_{33}H_{54}O_3$,

was isolated from the reaction mixture. The reactivity of the double bond was also shown by the ready bromination of cyclolaudenyl acetate to form cyclolaudenyl acetate dibromide, $C_{33}H_{54}O_2Br_2$. All of these products formed by the saturation of one ethylenic linkage showed the same pale yellow colour with tetranitromethane in chloroform and exhibited no selective absorption in the ultra-violet region.

The facts stated so far suggested a similarity between cyclolaudenol and cycloartenol in that the former might also have a tetracyclic triterpenoid skeleton containing a cyclopropane bridge and a reactive side chain double bond. Further evidence about the supposed side chain double bond was obtained by D.S.Irvine (74) by ozonolysis of cyclolaudenyl acetate with isolation of formaldehyde in good yield and a ketone, $C_{32}H_{52}O_3$, which gave a pale yellow tetranitromethane colour and no ultra-violet light absorption. Thus, in contrast to cycloartenol, cyclolaudenol was shown to have the functional group $>C=CH_2$, possibly in a side chain. Further evidence in favour of a cyclopropane bridge was obtained by treatment of cyclolaudanyl acetate (as for cycloartanyl acetate) with dry hydrogen chloride in chloroform. The product,

m.p.145-155°, showed a strong yellow tetranitromethane colour and had ultra-violet light absorption maximum at 2080 Å (ϵ = 3100). After seven crystallisations from chloroform-methanol pure laudenyl acetate, $C_{32}H_{50}O_2$, m.p.173-174°, was obtained in about 5 per cent yield. This compound, as described below, was prepared in greatly improved yield by another method. Further proof of a relationship between cycloartenol and cyclolaudenol was found in the comparison of the molecular rotation differences between the compounds and their derivatives which is set out in table F.

TABLE F

Compound	$[M]_D$ Alcohol	Acetate 1	Benzoate 2	Ketone 3	Differences Δ_1	Δ_2	Δ_3
cycloArtenol	+230°	+280°	+400°	+93°	+50°	+170°	-137°
cycloLaudenol	+206	+265	+343	+83	+59	+137	-123
cycloArtanol	+214	+277	-	+102	+63	-	-112
cycloLaudanol	+190	+242	-	-	+52	-	-

Barton(63) first pointed out the negative change in molecular rotation on oxidation of cycloartenol to cycloartenone. This is an unusual feature in triterpenoid

compounds and the only previously known tetracyclic triterpene which shared it was butyrospermol. Table F shows that in this and in the differences obtained on acetylation and benzoylation, cycloartenol and cyclolaudenol are very similar. Barton and Jones (87; cf. also 16) have observed that the tetracyclic triterpenes containing reactive double bonds form a single group, the members of which show little change in molecular rotation on hydrogenation - on the values given above, cyclolaudenol can be assigned to this group. A further conclusion can be drawn from the data in table F. Halsall, Meakins and Swayne (5) have observed that in triterpenes with a 3β-hydroxyl group the change in molecular rotation on acetylation, although variable in size, is positive. Compounds having a 3α-hydroxyl show a large negative change on acetylation, e.g. epihydroxyelemadienic acid gives a negative shift ($\Delta [M]_D$ -104°) as does polyporenic acid on acetylation ($\Delta [M]_D$ -200°) and benzoylation ($\Delta [M]_D$ -300°). Therefore, assuming that cyclolaudenol had the normal triterpene ring A, the configuration of the hydroxyl group was taken to be 3β.

The first possibility suggested by the known facts was that cycloartenol and cyclolaudenol might have the

same carbon skeleton and only differ in the location of the double bond in the side chain. However it was shown conclusively that cyclolaudanol and the derived acetate are not identical with the corresponding compounds from cycloartenol. At this stage it was considered important to prepare fully saturated compounds by cleavage of the cyclopropane ring followed by reduction in order to compare them with the corresponding lanosterol and euphol derivatives. In order to prepare the fundamental hydrocarbon laudane it was necessary to obtain laudanyl acetate in good yield from the hydrogen chloride rearrangement product of cyclolaudanyl acetate. This was done by a method similar to that used for the mixed acetates from cycloartanyl acetate. The crude rearrangement product, m.p.145-155°, was hydrogenated at 80°, with platinum catalyst, for 7 hours. The product, m.p.153-156°, which still gave a yellow tetranitromethane colour was treated with chromic acid in acetic acid under moderately vigorous conditions and the resulting material chromatographed. The fractions which were first eluted gave laudanyl acetate, $C_{33}H_{58}O_2$ in 50 per cent overall yield. This compound showed no tetranitromethane colour and no ultraviolet absorption. The oxidised fraction from the

chromatogram was set aside for further examination. Laudanyl acetate was hydrolysed to laudanol, $C_{31}H_{50}O$, which was oxidised by mild treatment with chromic acid to laudanone, $C_{31}H_{48}O$. Wolff-Kishner reduction of laudanone gave laudane, $C_{31}H_{50}$, in good yield. The non-identity of laudanone and laudane with the corresponding compounds in the lanosterol and euphol series meant that a new type of carbon skeleton for the laudenol series had to be considered and it was at this stage that the possibility of a C_{31} or C_{32} molecule was first seriously considered. Although laudenol had been shown to have a different skeleton from lanosterol a comparison of molecular rotation differences as set out in tables G and H indicated a close relationship between the two compounds.

TABLE G

$[M]_D$

Compound	Alcohol	Acetate 1	Ketone 3	Differences Δ_1	Δ_3
Lanostanol	+150°	+193°	+116°	+43°	-34°
Laudanol	+93	+155	+62	+62	-31

TABLE H

Compound	$[M]_D$	Compound	$[M]_D$	Difference
cycloArtanyl Acetate	+277°	Lanost-9(11)-enyl Acetate	+400°	+123°
cycloLaudanyl Acetate	+242	Laudenyl Acetate	+337	+145

In order to isolate laudenyl acetate from the crude product, m.p.145-155°, from cyclolaudanyl acetate on hydrogen chloride treatment, the procedure previously used to obtain lanost-9(11)-enyl acetate was adopted. The crude material, m.p.145-155°, was oxidised under very mild conditions with chromic acid in acetic acid and, by chromatography on alumina three compounds were isolated from the product. Laudenyl acetate was obtained in 35 per cent overall yield and was identical with the product isolated in 5 per cent yield by continued crystallisation of the product, m.p.145-155° (see above). The second compound was obtained in 10 per cent yield as yellow blades, m.p.186°; it had empirical formula, $C_{33}H_{52}O_4$, gave no colour with tetranitromethane and had a light absorption maximum at 2700 Å (ϵ = 7400). This compound was also isolated from the oxidised

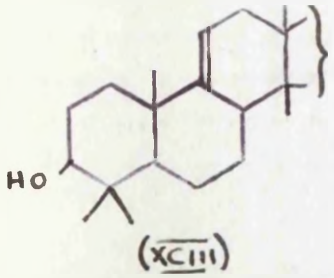
fraction in the experiment (described above) in which laudenyl acetate was prepared from the mixed acetates, m.p.145-155°. The third compound, m.p.192-193°, was obtained in small yield, gave no colour with tetranitromethane and had an ultra-violet light absorption maximum at 2410 Å (ϵ = 11,400); it was prepared in good yield by oxidation of laudenyl acetate with chromic acid in refluxing acetic acid. Analysis corresponded to a formula, $C_{33}H_{54}O_3$.

The remarkably close analogy of the foregoing reactions with those of cycloartenol and its derivatives, strongly suggested that cycloartenol and cyclolaudenol have an identical pentacyclic nucleus, as had already been postulated on the basis of molecular rotation differences. This implies that laudenol has the nuclear structure of lanost-9(11)-enol and can be represented by part structure (XCIII). The yellow compound, m.p.186°, which has ultra-violet light absorption characteristics identical with those of 7:11-dioxolanost-8-enyl acetate was therefore named 7:11-dioxoland-8-enyl acetate and represented by part structure (XCIV). Oxidation of laudenyl acetate gave the compound described above having an ultra-violet spectrum characteristic of a $\beta\beta$-disubsti-

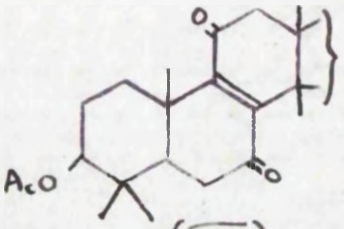
tuted αβ-unsaturated ketone, in the same way as lanost--9(11)-enyl acetate gave 12-oxolanost-9(11)-enyl acetate. Therefore the compound was named 12-oxolaud-9(11)-enyl acetate (XCV). Further, by treatment with zinc dust in acetic acid, 7:11-dioxolaud-8-enyl acetate was reduced to 7:11-dioxolaudanyl acetate, $C_{33}H_{54}O_4$ (XCVI) which gave no colour with tetranitromethane and showed no selective absorption in the ultra-violet in direct analogy to the preparation (35) of 7:11-dioxolanostanyl acetate from 7:11-dioxolanost-8-enyl acetate. Again, the transformation of laudenol derivatives brought about changes in molecular rotation similar to those in the lanosterol series - comparisons of these values are shown in table I.

TABLE I

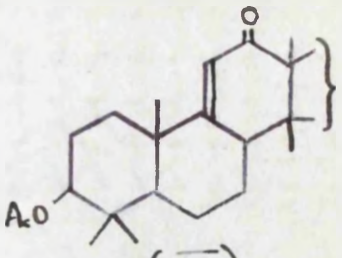
Compound	$[M]_D$	Compound	$[M]_D$	Difference
Lanost-9(11)-enyl acetate	+400°	12-Oxolanost-9(11)--enyl acetate	+440°	+40°
Laud-9(11)-enyl acetate	+337	12-Oxolaud-9(11)--enyl acetate	+434	+47
7:11-Dioxolanost--8-enyl acetate	+460	7:11-dioxolanost-anyl acetate	+320	-140
7:11-Dioxolaud-8--enyl acetate	+364	7:11-Dioxolaudanyl acetate	+257	-107



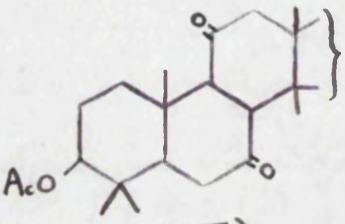
(XCIII)



(XCIV)



(XCV)

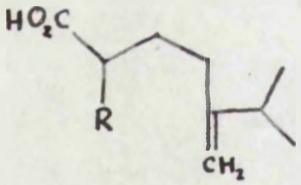


(XCVI)

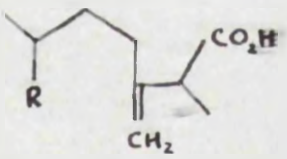
By the kind co-operation of Dr. A.R.H. Cole, University of Western Australia, confirmatory evidence of the close relationship of cyclolaudenol to cycloartenol was obtained. cycloLaudenol, cyclolaudenyl acetate and cyclolaudanyl acetate in carbon tetrachloride solution all showed the infra-red absorption band at 3040 cm.$^{-1}$ which had previously been observed for cycloartenol, cycloartanol and i-cholestane (see Theoretical, Part I) and attributed to the presence of a CH_2 group in a three membered ring. The infra-red spectra of cyclolaudenol and its acetate also showed bands at 3071 cm.$^{-1}$ (carbon tetrachloride) and 887 cm.$^{-1}$ (carbon disulphide) indicating the presence of the group $>C=CH_2$ which had already

been shown by chemical methods.

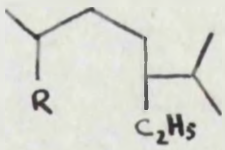
The fact that laudane was not identical with euphane or lanostane indicated a less common carbon skeleton for laudenol and, as previously mentioned, raised the possibility of a C_{31} or C_{32} formula. The recent work reviewed in the historical part of this thesis, on the elucidation of the structures of eburicoic acid and the polyporenic acids A, B and C made the idea of a C_{31} formula attractive, especially since the fundamental differences between these compounds and lanosterol lie in the side chain and not in the nucleus. Eburicoic acid and polyporenic acids B and C have the structure (XCVII) and polyporenic acid A the structure (XCVIII) in the side chain.



(XCVII)



(XCVIII)

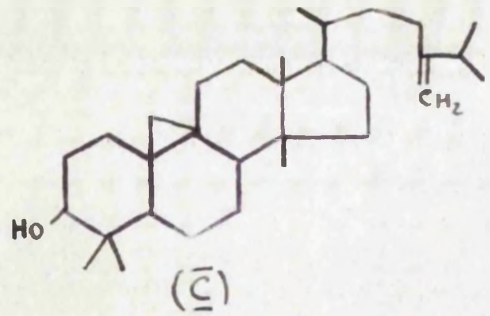


(IC)

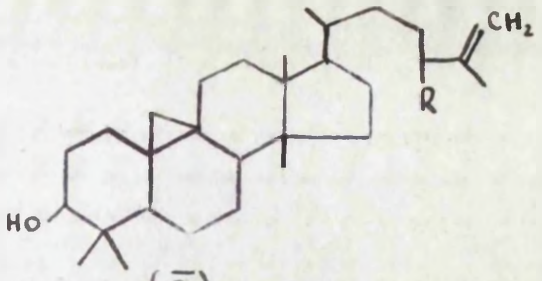
Thus the carbon atom arrangement found in the side chain of ergostanol which is common to all the known C_{31} triterpenoids was considered possible for cyclolaudenol. However, a side chain of the stigmasterol type (IC), although C_{32} triterpenoids having such a structure had

not previously been described, was also considered.

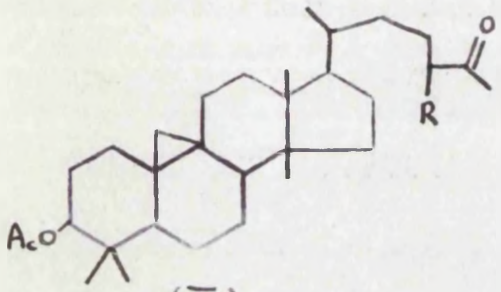
Since all the established C_{31} triterpenoids were known to have the side chain double bond between $C_{(24)}$ and $C_{(28)}$ it was suspected that cyclolaudenol might also have this structure, i.e. (C). However, D.S.Irvine (74) by Wolff-Kishner reduction of ''norcyclolaudanonyl acetate'', (24-oxonorcyclolaudanyl acetate) the previously described ozonolysis product of cyclolaudenyl acetate, obtained norcyclolaudanyl acetate which was different from cycloartanyl acetate. Further, the derived norcyclolaudanol and norcyclolaudanone were not identical with cycloartanol and cycloartanone respectively. It was subsequently shown (74) that norcyclolaudanonyl acetate is a methyl ketone by oxidation with potassium hypobromite (bromoform test) to give, in smallyield, an acid, bisnorcyclolaudanoloic acid acetate. On this evidence the structure (CI) was suggested for cyclolaudenol and, therefore, structures (CII) and (CIII) for norcyclolaudanonyl acetate and bisnorcyclolaudanoloic acid acetate respectively - in all these R was considered to be CH_3 or C_2H_5.



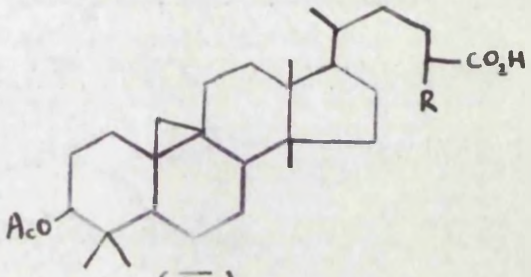
(C)



(C I)



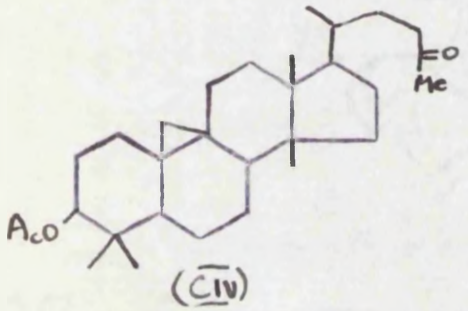
(C II)



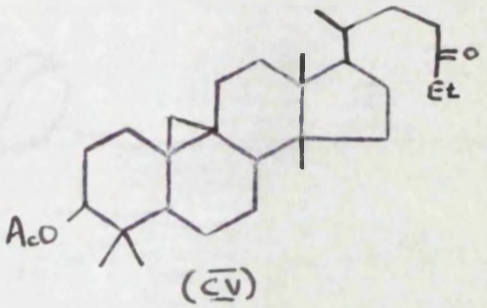
(C III)

While the stepwise degradation (described below) of the side chain of cyclolaudenol was undertaken by D.S.Irvine, 24-oxobisnorcycloartanyl acetate (CIV) and 24-oxonorcycloartanyl acetate (CV) were prepared starting from cycloartenyl acetate. It was expected that one of these compounds would be obtained from cyclolaudenol. By ozonolysis of cycloartenyl acetate in dry chloroform at -45° trisnorcycloartan-24-aldehyde acetate (CVI) was obtained in 75 per cent yield. The aldehyde was characterised by preparation of an oxime and by preparation of the dimethoxy compound (CVII) which formed simply on solution in methanol. The methyl (CIV) and ethyl

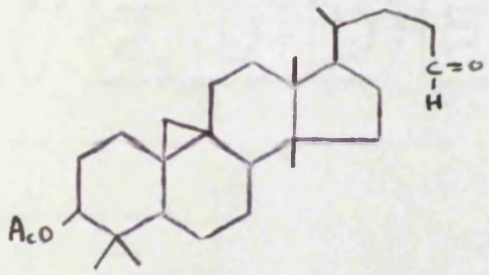
ketones (CV) were prepared by treatment of the trisnor-aldehyde in dry ether and dioxan with diazomethane and diazoethane respectively. These compounds were characterised by preparation of oximes and were shown to be ketones by their stability to chromic acid in acetic acid.



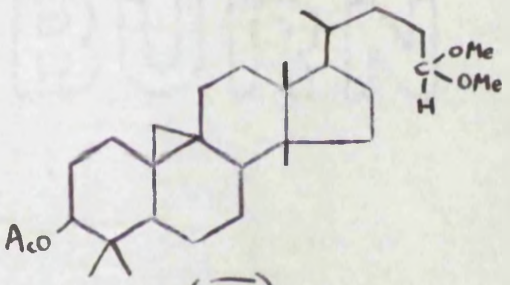
(CIV)



(CV)



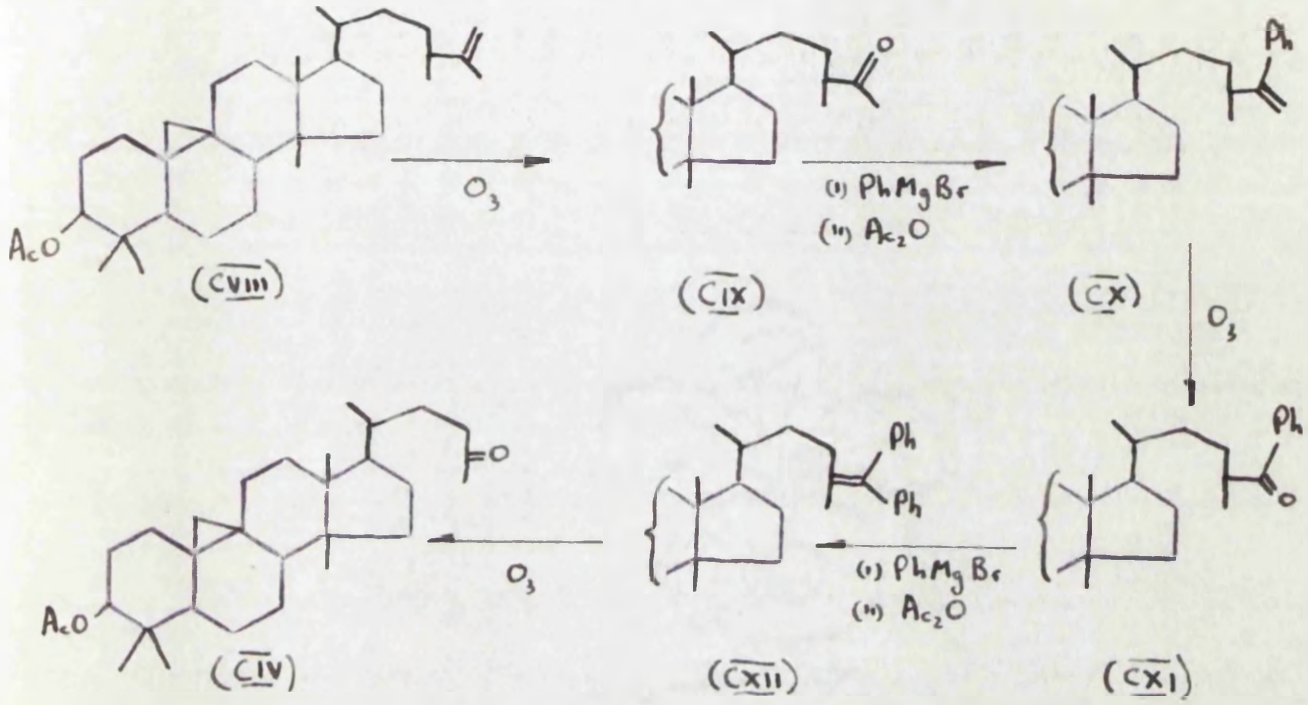
(CVI)



(CVII)

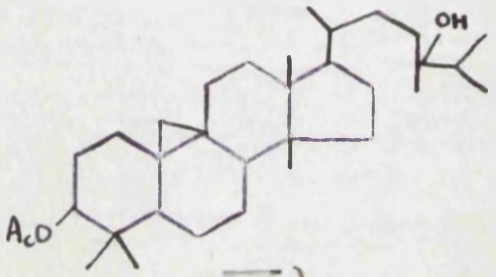
By degradation of cyclolaudenyl acetate, D.S.Irvine (74) obtained a methyl ketone having the same constants as the methyl ketone described above, and giving no melting point depression when mixed with it. Thanks to the co-operation of Professor E.R.H. Jones, the infra-red spectra of these compounds were compared and shown to be identical. The steps involved in the preparation of

24-oxobisnorcycloartanyl acetate (CIV) from cyclolaudenyl acetate (CVIII) are outlined in the scheme below.



An attempt was made to prepare cyclolaudanyl acetate from 24-oxobisnorcycloartanyl acetate. The methyl ketone was treated with isopropylmagnesium bromide and the product, without purification was acetylated, and then dehydrated by means of phosphorus oxychloride in pyridine to give, in about 10 per cent yield, a crystalline product, m.p.130-150°, showing a strong yellow colour with tetranitromethane. The low yield can be attributed to dehydration conditions having been unnecessarily vigorous. The product, of wide melting range,

was probably a mixture of double bond isomers, the intermediate (CXIII) not having dehydrated stereospecifically. Hydrogenation of this product in presence of platinum catalyst gave an apparently pure acetate, $C_{33}H_{56}O_2$, which was not identical with cyclolaudanyl acetate. This compound gave a pale yellow colour with tetranitromethane and showed no selective absorption in the ultra-violet region.



(CXIII)

The difference between the two acetates must lie in the configurations about $C_{(24)}$; the partly synthesised acetate, $C_{33}H_{56}O_2$, having the $C_{(24)}$ methyl group in the opposite configuration to cyclolaudanyl acetate or being a mixed crystal of the two epimers. D.S.Irvine (74) observed that compounds (CIX) and (CXI) which have carbonyl groups adjacent to $C_{(24)}$ readily isomerise to mixtures of epimers. The diastereoisomers formed by norcyclolaudanonyl acetate (CIX) could not be separated

but normal- and epi-forms of the derived styryl compound (CX) and of the phenyl ketone (CXI) were isolated.

cyclolaudenol and its derivatives, therefore, cannot at this stage be named as compounds of the eburicane series as polyporenic acid C and the eburicoic acid have been. Both polyporenic acid C (20) and eburicoic acid (33) on hydrogenation of the 24(28) double bond reacted in a stereospecific manner to give dihydro-compounds having the same configuration of the $C_{(24)}$ methyl group. Since no corresponding side chain saturated compounds which could be compared have been made from cyclolaudenol and polyporenic acid C or eburicoic acid, it is not known if the hypothetical eburicane has the same configuration of the $C_{(24)}$ methyl group as laudane, and is therefore identical with it. The 24(28) double bond in polyporenic acid A, however, hydrogenates (16) to give a mixture of the $C_{(24)}$ configurations, and two series of $C_{(24)}$ epimers have been separated. It appears, therefore, that it would be most profitable to attempt interconversion between these two series, and a derivative of cyclolaudenol.

EXPERIMENTAL

Melting points are uncorrected.

Specific rotations were measured in chloroform solution in a 1 dm. tube at room temperature.

Ultra-violet absorption spectra were measured in absolute ethanol solution using a Unicam SP.500 spectrophotometer.

Grade II alumina and a light petroleum fraction of b.p.60-80° were used for chromatography unless otherwise specified.

''Stabilised acetic acid'' denotes acetic acid which has been refluxed over and distilled from chromic anhydride.

The analysts were Dr. A.C. Syme and Mr. W. McCorkindale of The Royal Technical College, Glasgow.

Hydrolysis of S.Nuxvomica Seed Fat. - A solution of sodium hydroxide (160 g.) in water (200 ml.) was added to a boiling solution of the brown, semi-solid fat (1 kg.) in ethanol (3 l.) and the mixture refluxed for 4 hours, concentrated to 1.5 litres and mixed with hot water (12 l.) The resulting suspension was cooled and extracted with ether (5 x 2.5 l.). After being washed with water the combined extracts were evaporated and the product freed from water by co-distillation using benzene. The non-

-saponifiable fraction (125 g.) was obtained as an orange--brown, waxy solid.

Chromatography of Acetylated Non-saponifiable Fraction. - The non-saponifiable matter (90 g.) in dry pyridine (250 ml.) and acetic anhydride (100 ml.) was kept at room temperature for 24 hours. The mixture was poured into water, the product extracted in ether and the ether extract washed with N hydrochloric acid, water, and dried (Na_2SO_4). The acetylated product formed an orange-coloured solid (96 g.), a solution of which in light petroleum (1 l.) was percolated through a column (132 cm. x 5.5 cm.) of alumina (3 kg.). Eluant fractions of 1 litre were collected and the chromatogram developed as shown.

Fraction	Eluant	Weight	Description	M.p.
1	Light petroleum	-	-	-
2	''	Trace	Colourless oil	
3	''	0.74 g.	''	
4	''	1.41	''	
5	''	3.21	''	
6	Light petroleum: Benzene (1:1)	1.15	''	

Fraction	Eluant	Weight	Description	M.p.
7	Light petroleum: Benzene (1:1)	Trace	Colourless gum	
8	''	13.05 g.	White solid	217-219°
9	''	12.16	''	216-217
10	''	7.09	''	212-214
11	''	4.17	White solid and colourless gum	
12	''	2.49	Colourless gum	
13	Benzene	2.00	''	
14	''	1.50	''	
15	Benzene:Ether (9:1)	3.00	Yellow gum	110-112
16	''	2.00	''	110-111
17	''	2.52	Yellow gum and crystals	115-118
18	''	16.15	Pale yellow solid	113-119
19	''	2.32	Yellow gum and crystals	97-109
20	''	0.35	Yellow gum	
21	''	Trace	''	

The column was finally eluted with acetone (6 l.) to give a brown viscous oil (16.34 g.). This fraction was rechromatographed by M.B.E. Fayez (61) and stigmasteryl acetate, m.p.142.5-143°, isolated.

Fractions (2-6) were combined. This colourless oil with a faint lemon odour has not been further examined.

α-Amyrin Acetate. - Fractions (8-10) of the foregoing chromatogram were combined and crystallised several times from chloroform-methanol to give α-amyrin acetate (30.4 g.) as elongated plates, m.p. alone and mixed with an authentic specimen, 223-225°, $[\alpha]_D$ +79.5° (c, 1.6), giving a pink Liebermann-Burchardt reaction and a strong yellow colour with tetranitromethane.

Found: C,81.8; H,11.2

Calc. for $C_{32}H_{52}O_2$: C,82.0; H,11.2%.

Hydrolysis of acetate (0.5 g.) by refluxing in ethanolic potassium hydroxide solution (100 ml., 3%) for 4 hours gave, by the usual isolation procedure, α-amyrin which crystallised from aqueous methanol as needles (0.4 g.), m.p. and mixed m.p.185°, $[\alpha]_D$ +82° (c, 2.0).

Found: C,84.5; H,11.9

Calc. for $C_{30}H_{50}O$: C,84.4; H,11.8%.

cycloArtenyl Acetate. - Fractions (15-18) (above) were combined, several crystallisations from chloroform--methanol giving cycloartenyl acetate (12 g.) as irregular plates, m.p.122-124°, $[\alpha]_D$ +59.5° (c, 1.8), showing

a strong yellow colour with tetranitromethane in chloroform.

Light absorption in ethanol: Max. at 2090 Å (ϵ = 1480).

Found: C,81.9,81.8; H,11.4,11.2

Calc. for $C_{32}H_{52}O_2$: C,82.0; H,11.2%.

In the Liebermann-Burchardt test the compound gave a yellow solution which developed a blood red colour having a green fluorescence. Professor D.H.R. Barton found that mixture with cycloartenyl acetate, m.p.121.5-122.5°, from Artocarpus integrifolia had m.p.121.5-122.5°.

cycloArtenol. - A solution of cycloartenyl acetate (820 mg.) in 10% ethanolic potassium hydroxide (25 ml.) was refluxed for 4 hours, cooled and poured into water. The ether extract was washed with water and dried (Na_2SO_4). Crystallisation of the product from methanol gave cycloartenol (700 mg.) as fine needles, m.p.99°, raised to 115° after drying for 12 hours at 65° in vacuo. $[\alpha]_D$ +54° (c, 1.3).

Found: C,84.5; H,11.8

Calc. for $C_{30}H_{50}O$: C,84.4; H,11.8%.

Professor D.H.R. Barton found that mixture with a solvated sample of cycloartenol, m.p.110°, from Artocarpus integrifolia had m.p.114.5-115.5°.

cycloArtenyl Benzoate. - A solution of cycloartenol (440 mg.) in dry pyridine (5 ml.) was treated with benzoyl chloride (0.15 ml.) and heated on the steam bath for 4 hours. The reaction mixture was cooled, diluted with ether, washed with 3N hydrochloric acid, 2% aqueous sodium hydroxide, water and dried (Na_2SO_4). The product was crystallised from methanol to give cycloartenyl benzoate (400 mg.) as needles, m.p.130°, $[\alpha]_D$ +76° (c,0.5; 0.8).

Found: C,83.5; H,10.25

Calc. for $C_{37}H_{54}O_2$: C,83.7; H,10.25%.

cycloArtenone. - A solution of cycloartenol (600 mg.) in stabilised acetic acid (60 ml.) was treated, at room temperature, with a solution of chromic acid (103 mg.) in stabilised acetic acid (10 ml.) added dropwise during 30 minutes with stirring. The solution was kept at room temperature overnight, methanol added and the mixture poured into water. The ether extract was washed with 10% aqueous sodium bicarbonate, water and dried (Na_2SO_4). The product crystallised from methanol as plates, m.p.102-105°. Concentration of the mother liquor gave a second crop of plates, m.p.95°. Further crystallisation of the first crop from methanol gave cycloartenone as plates, m.p.105-

-106°, $[\alpha]_D$ +22° (c, 1.1).

Light absorption in ethanol: Max. at 2120 Å (ϵ = 1470).

Found: C,85.0; H,11.35

Calc. for $C_{30}H_{48}O$: C,84.8; H,11.4%.

cycloArtanyl Acetate. - cycloArtenyl acetate (1 g.) in stabilised acetic acid (370 ml.) was added to platinum catalyst (from 0.5 g. platinum oxide) in acetic acid (30 ml.). Shaking with hydrogen was carried out at room temperature and atmospheric pressure for 2 hours. The solution was filtered and evaporated under reduced pressure. Crystallisation of the residue from chloroform--methanol yielded long needles (0.9 g.), m.p.130-132°. Further crystallisation from chloroform-methanol gave cycloartanyl acetate as long needles, m.p.130-132°, $[\alpha]_D$ +59° (c, 1.9; 1.7).

The compound gave a plae yellow colour with tetranitromethane in chloroform and showed no selective light absorption in the region 2000-4000 Å.

Found: C,81.8; H,11.8

Calc. for $C_{32}H_{54}O_2$: C,81.6; H,11.7%.

cycloArtanol. - A solution of cycloartanyl acetate (300 mg.) in 3% methanolic potassium hydroxide (25 ml.)

and benzene (2 ml.) was refluxed for 3 hours, cooled and poured into water. The ether extract was washed with water and dried (Na_2SO_4). The product crystallised from methanol to give cycloartanol as plates (200 mg.), m.p.99° raised to 106° after drying for 30 hours at 78° in vacuo, $[\alpha]_D$ +50° (c, 0.7).

Found: C,84.1; H,12.3

Calc. for $C_{30}H_{52}O$: C,84.0; H,12.2%.

cycloArtanone. - A solution of chromic acid (125 mg.) in stabilised acetic acid (10 ml.) was added dropwise with stirring during 1 hour to a solution of cycloartanol (660 mg.) in stabilised acetic acid (70 ml.). The solution was kept at room temperature overnight, methanol added and the mixture poured into water. The ether extract was washed with 10% aqueous sodium bicarbonate, water and dried (Na_2SO_4). The product crystallised from methanol as plates (510 mg.), m.p.109-110°. Further crystallisation from methanol gave cycloartanone as elongated plates, m.p.110°, $[\alpha]_D$ +24° (c, 2.1, 2.9). The compound gave a pale yellow colour with tetranitromethane in chloroform and showed no selective absorption in the ultra-violet.

Found: C,84.8; H,11.9

$C_{30}H_{50}O$ requires: C,84.4; H,11.8%.

cycloArtanone (100 mg.) in methanol (15 ml.) was added to a solution of semicarbazide hydrochloride (300 mg.) and sodium acetate (200 mg.) in methanol (10 ml.) and the minimum of water. The product separated as small needles (100 mg.), m.p. 215° (decomp.). Further crystallisation from methanol gave the semicarbazone as needles, m.p. 215° (decomp.).

Found: C, 76.9; H, 10.6

$C_{31}H_{53}ON_3$ requires: C, 77.0; H, 11.0%.

Treatment of cycloArtenyl and cycloArtanyl Acetate with Perbenzoic Acid. - To cycloartenyl acetate (500 mg.) in chloroform at 0° was added in one portion a chloroform solution of perbenzoic acid (20 ml., 48 mg./ml., 6.5 mol.) at 0°. An exactly similar solution of cycloartanyl acetate and a blank solution were made up. The three solutions were maintained at 0°. After total times of 16 hours and 64 hours, samples (2 ml.) were drawn from each reaction and titrated with standard sodium thiosulphate solution to give the results shown.

	Perbenzoic acid reacted	
	16 hours	64 hours
cycloArtenyl acetate	1.01 mol.	1.01 mol.
cycloArtanyl acetate	0	0

cycloArtenyl Acetate Oxide. - To cycloartenyl acetate (500 mg.) in chloroform (10 ml.) at 0° a chloroform solution of perbenzoic acid (1.8 ml., 104 mg./ml., 1.2 mol.) was added in one portion. The reaction was kept at 0° for 24 hours, then diluted with chloroform and washed with 10% aqueous sodium thiosulphate, 10% aqueous sodium hydroxide, water and dried (Na_2SO_4). Chloroform was evaporated, the residue dissolved in benzene (100 ml.) and the solution percolated through a column of alumina (10 g.). The column was further eluted with benzene (200 ml.) to give a product which separated from methanol as a crystalline powder (260 mg.), m.p.135-140°. Further crystallisation from methanol gave cycloartenyl acetate oxide as short rods, m.p.144°, $[\alpha]_D$ +55°, +57° (c, 1.7, 2.0). The compound gave a pale yellow colour with tetranitromethane and showed no selective light absorption in the region 2000-4000 Å.

Found: C,79.5; H,11.2

$C_{32}H_{52}O_3$ requires: C,79.3; H,10.8%.

cycloArtenyl Acetate Dibromide. - cycloArtenyl acetate (500 mg.) in glacial acetic acid (100 ml.) was treated at room temperature with a solution of bromine (206 mg., 1.3 mol) in glacial acetic acid (20 ml.)

added dropwise. The solvent was evaporated under reduced pressure at 30° and the residue crystallised from chloroform-methanol as small plates (430 mg.), m.p.135-155°. Several crystallisations from chloroform-methanol gave cycloartenyl acetate dibromide as small plates, m.p.163--167°, $[\alpha]_D$ +33°, +39° (c, 1.1, 1.3). The compound gave a light yellow colour with tetranitromethane in chloroform and showed no selective absorption in the region 2000--4000 Å.

Found: C,61.7; H,8.5

$C_{32}H_{52}O_2Br_2$ requires: C,61.1; H,8.3%.

Treatment of cycloArtanyl Acetate with Chromic Acid.- A solution of cycloartanyl acetate (1.4 g.) in stabilised acetic acid (100 ml.) at 70° was treated with a solution of chromic acid (0.9 g. ≡ 4.5 O) in stabilised acetic acid (25 ml.) added dropwise with stirring during 1 hour. The solution was stirred for a further 3 hours at 70° and then kept overnight at room temperature. Methanol (5 ml.) was added and the mixture poured into water. The ether extract was washed with 10% sodium carbonate solution, water and dried (Na_2SO_4). The product was crystallised from methanol as needles (1 g.), m.p.123-130°, giving a pale yellow colour with tetranitromethane and no selective

ultra-violet light absorption. The compound showed no depression in melting point when mixed with starting material.

Treatment of cycloArtanyl Acetate with Sulphuric Acid. - (a) cycloArtanyl acetate (200 mg.) in purified dioxan (50 ml.) was treated with concentrated sulphuric acid (1 ml.) and water (0.5 ml.). The homogeneous solution was kept at room temperature for 7 days and then poured into water. The ether extract was washed with 10% sodium carbonate, water and dried (Na_2SO_4). The product crystallised from chloroform-methanol as needles (160 mg.), m.p.131° alone or mixed with starting material, $[\alpha]_D$ +59° (c, 2.0).

(b) To cycloartanyl acetate (200 mg.) in ethanol (75 ml.) was added a solution of concentrated sulphuric acid (2 ml.) in water (3 ml.) and ethanol (10 ml.). The solution was refluxed for 3 hours, cooled and worked up as for (a). The product was dissolved in dry pyridine (10 ml.) and acetic anhydride (2 ml.), heated on the steam bath for 30 minutes and worked up in the usual manner to give, by crystallisation from methanol, long needles (150 mg.), m.p. 130° alone or mixed with starting material, $[\alpha]_D$ +58° (c, 1.5).

Treatment of cycloArtanyl Acetate with Boron Trifluoride. - Redistilled boron trifluoride etherate (2mL) was added to cycloartanyl acetate (200 mg.) in dry benzene (25 ml.) and the solution kept at room temperature for 6 days. The mixture was diluted with ether, washed with 10% aqueous sodium bicarbonate, water and dried (Na_2SO_4). The product crystallised from chloroform--methanol as needles (165 mg.), m.p.130° alone or mixed with starting material, $[\alpha]_D$ +59.5° (c, 1.3).

Isomerisation of cycloArtanyl Acetate. - cycloArtanyl acetate (500 mg.) in dry chloroform (20 ml.) was externally ice-cooled and treated with a vigorous stream of dry hydrogen chloride for 3 hours. The solvent was evaporated under reduced pressure at 30° and the residue crystallised from chloroform-methanol as plates and needles (450 mg.), m.p.137-157°, $[\alpha]_D$ +68° (c, 2.4). The material gave a strong yellow colour with tetranitromethane in chloroform. Light absorption in ethanol: Max. at 2070 Å (ϵ = 3600). Repeated crystallisation from the same solvent was accompanied by a gradual and continuous increase in melting point and rotation and considerable loss of material. After the fifth crystallisation the material separated as blades (50 mg.), m.p.157-159°,

$[\alpha]_D$ +76° (c, 1.7). Alkaline hydrolysis of the acetate mixture, m.p.137-157°, gave an alcohol mixture which crystallised from methanol as needles, m.p.152-154°, $[\alpha]_D$ +60° (c, 1.5). Reacetylation gave an acetate, m.p.137--160°. Oxidation of the alcohol mixture, m.p.152-154°, with chromic acid in acetic acid at room temperature gave a ketone mixture which crystallised from methanol as large plates, m.p.121-123°, $[\alpha]_D$ +37° (c, 2.9). Careful chromatographic examinations of the acetate mixture, m.p. 137-157°, and the ketone mixture, m.p.121-123°, showed no separation of isomers. By treatment of lanost-8-enyl acetate with hydrogen chloride in chloroform (73) the equilibrium mixture of lanost-7- and -8-enyl acetates was obtained and crystallised from chloroform-methanol as plates, m.p.127-134°, $[\alpha]_D$ +50° (c, 2.1). This material (40 mg.) was mixed with lanost-9(11)-enyl acetate (60 mg.) and crystallised from chloroform-methanol as plates, m.p.138-158°, $[\alpha]_D$ +73° (c, 2.1) which melted over the same range when mixed with a sample of the acetate mixture from cycloartanyl acetate. Hydrolysis gave an alcohol mixture, $[\alpha]_D$ +63° (c, 1.8), m.p.153-154° alone or mixed with the alcohol, m.p.152-154°, above.

Lanost-9(11)-enyl Acetate. - (a) A solution of chromic acid (1.2 g. ≡ 4 O) in stabilised acetic acid (50 ml.) was added during 1 hour with stirring to a refluxing solution of the acetate mixture (m.p.137-157°, 2 g.) in stabilised acetic acid (100 ml.). The solution was refluxed for a further 2 hours and then kept at room temperature overnight. Methanol was added and solvent evaporated under reduced pressure. The ether extract of the residue was washed with 10% aqueous sodium carbonate, water and dried (Na_2SO_4). A solution of the yellow product (1.8 g.) in light petroleum (50 ml.) was percolated through a column (2 cm. x 23 cm.) of alumina (60 g.), and the chromatogram eluted with light petroleum, light petroleum-benzene (9:1, 4:1, 7:3, 1:3), benzene and benzene-ether (9:1). Fractions (10-25)(0.21 g.) eluted by light petroleum-benzene (4:1, 800 ml.) were combined and crystallised from chloroform-methanol as hexagonal plates (0.18 g.), m.p.167-170°. Further crystallisation from the same solvent gave lanost-9(11)-enyl acetate as hexagonal plates, m.p.170-171°, $[\alpha]_D$ +87° (c, 0.9). When mixed with the specimen prepared from 7:11-dioxolanost--8-enyl acetate (see below) the melting point was undepressed. The compound gave a strong yellow colour with tetranitro-

methane in chloroform. Light absorption in ethanol: Max. at 2060 Å (ϵ = 4310).

Found: C,81.5; H,11.8.

Calc. for $C_{33}H_{54}O_2$: C,81.6; H,11.7%.

Fractions (29-63) eluted by light petroleum-benzene (7:3, 900 ml.; 1:1, 150 ml.; 1:3, 300 ml.) and benzene (300 ml.) yielded no homogeneous compound. Small amounts of amorphous material were obtained which showed light absorption at 2700 Å.

Fractions (65-68) (0.3 g.) eluted by benzene-ether (9:1, 200 ml.) were crystallised from methanol as needles (0.18 g.), m.p.175-180°. Further crystallisation from methanol gave the product as needles, m.p.183-185°, $[\alpha]_D$ +94° (c, 1.0). Light absorption in ethanol: Max. at 2400 Å (ϵ = 9850). When mixed with a specimen of 12-oxolanost-9(11)-enyl acetate prepared from lanost--9(11)-enyl acetate (see below) the melting point was undepressed.

(b) A solution of chromic acid (0.3 g.) in 90% acetic acid (25 ml.) was added during 5 minutes with stirring to a solution of the acetate mixture (m.p.137-157°, 1.0 g.) in acetic acid (75 ml.) heated on the steam bath. Heating was continued for 10 minutes, the mixture was

poured into water and the solid collected by means of ether. The ether extract was worked up as in (a) above and the dry product (1.1 g.) dissolved in light petroleum (100 ml.) was percolated through a column (2 cm. x 23 cm.) of alumina (60 g.). The chromatogram was developed by elution with light petroleum and light petroleum-benzene (9:1 then 4:1). The last solvent (650 ml.) eluted a fraction (448 mg.), m.p.167-170°, which on crystallisation from chloroform-methanol gave lanost-9(11)-enyl acetate as hexagonal plates, m.p.170-172°, $[\alpha]_D$ +85° (c, 1.6). The product was identical with that obtained by method (a) above.

Hydrogenation of ''isocholesteryl'' Acetate. - ''isocholesteryl'' acetate (m.p.125°, 30 g.) in glacial acetic acid (330 ml.) was shaken with hydrogen for 4 hours at 70° in presence of platinum catalyst (previously reduced from 2 g. platinum oxide). The solution was filtered and evaporated under reduced pressure. The product crystallised from chloroform-methanol as long blades (25g.) m.p.118-120°.

7:11-Dioxolanost-8-enyl Acetate. - (cf. 33). The hydrogenated product (20 g.) from the foregoing experiment

was dissolved in stabilised acetic acid (1 l.). The solution was stirred on the steam bath and chromic acid (13 g.) in water (30 ml.) was added during 30 minutes. Heating was continued for 1.5 hours, the solution cooled and poured into ice water. The solid product was coagulated by addition of sodium chloride, filtered, washed with water and dissolved in ether. The ether solution was washed with 5% aqueous sodium hydroxide, water and dried (Na_2SO_4). The dry product dissolved in benzene (250 ml.) was filtered through a short column of alumina (200 g.) and the column further eluted with benzene. The eluted material crystallised from methanol as yellow plates (12.5 g.), m.p.151-154°. Further crystallisation from methanol gave 7:11-dioxolanost-8-enyl acetate as yellow plates, m.p.156-158°, $[\alpha]_D$ +92° (c,1.2). Light absorption in ethanol: Max. at 2700 Å (ϵ = 7579).

Found: C,77.0; H,10.4.

Calc. for $C_{32}H_{50}O_4$: C,77.1; H,10.1%.

7:11-Dioxolanostanyl Acetate.-(35) Zinc dust (75 g.) was added in portions during 15 minutes to 7:11-dioxolanost-8-enyl acetate (12 g.) in boiling glacial acetic acid (500 ml.). Heating was continued for 1 hour, the

acetic acid decanted and the zinc further extracted with boiling acetic acid (250 ml.). The combined extracts were concentrated, poured into water and the solid collected in ether. The ether extract was washed with 10% aqueous sodium carbonate, water and dried (Na_2SO_4). The product crystallised from chloroform-methanol as plates (7.5 g.), m.p.220-221°. Further crystallisation from the same solvent gave 7:11-dioxolanostanyl acetate as plates, m.p.220-222°, $[\alpha]_D$ +57° (c, 1.6). The compound gave no colour with tetranitromethane in chloroform and showed no selective absorption in the ultra-violet.

Found: C,77.1; H,10.4

Calc. for $C_{32}H_{52}O_4$: C,76.8; H,10.4%.

11-Oxolanostanyl Acetate (20). - A solution of 7:11-dioxolanostanyl acetate (7.5 g.) in redistilled diethylene glycol (250 ml.) was heated with 100% hydrazine hydrate (3.8 ml.) at 200° for 1 hour. The mixture was cooled, then treated at 220-230° for 6 hours with diethylene glycol (70 ml.) to which sodium (7.5 g.) had been added. After cooling, the mixture was poured into water, acidified with hydrochloric acid and extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4), and evaporated to give a yellow solid (7 g.) which was

dissolved in pyridine (50 ml.) and acetic anhydride (50 ml.) and heated on the steam bath for 3 hours. The product (6.5 g.), isolated in the usual way, was dissolved in light petroleum-benzene (9:1, 100 ml.) and percolated through a column (1.5 cm. x 20 cm.) of alumina (30 g.). Further elution with the same solvent (150 ml.) gave a fraction (5.5 g.), m.p.138-140°. By recrystallisation from chloroform-methanol, 11-oxolanostanyl acetate was obtained as needles, m.p.143-144°, $[\alpha]_D$ +63.7° (c,1.1).

Found: C,79.2; H,11.4.

Calc. for $C_{32}H_{54}O_3$: C,79.0; H,11.2%.

11-Hydroxylanostanyl Acetate (28). - 11-Oxolanostanyl acetate (2.4 g.) in dry ether (100 ml.) was added to lithium aluminium hydride (2.5 g.) in dry ether (150 ml.); the mixture was refluxed for 2.5 hours, cooled and diluted with ether. Excess hydride was decomposed by cautious addition of water. The suspension was washed with 5N sulphuric acid, water and dried (Na_2SO_4). Ether was evaporated and the residue crystallised from chloroform--methanol as needles (1.5 g.), m.p.190-191°. This material in dry pyridine (20 ml.) and acetic anhydride (20 ml.) was kept overnight at room temperature and then worked up in the usual way to give 11-hydroxylanostanyl

acetate which crystallised from chloroform-methanol as needles, m.p.216°, $[\alpha]_D$ +22° (c,2.0).

Found: C,78.8; H,11.5

Calc. for $C_{32}H_{52}O_3$: C,78.6; H,11.2%.

Lanost-9(11)-enyl Acetate. - 11-Hydroxylanostanyl acetate (650 mg.) in dry pyridine (40 ml.) was treated with phosphorus oxychloride (5 ml.) and the mixture heated on the steam bath for 3 hours, cooled and poured into water. The ether extract was washed with 3N sulphuric acid, 10% sodium carbonate solution, water and dried (Na_2SO_4). A solution of the product (640 mg.) in light petroleum (100 ml.) was percolated through a column (1.5 cm. x 16 cm.) of alumina (30 g.) which was eluted with light petroleum and light petroleum-benzene (9:1, then 4:1). The last solvent (450 ml.) eluted a fraction (500 mg.) which was crystallised from chloroform-methanol to give lanost-9(11)-enyl acetate as hexagonal plates, m.p.173-174°, $[\alpha]_D$ +84° (c, 1.3). The compound gave a strong yellow colour with tetranitromethane in chloroform. Light absorption in ethanol: Max. at 2060 Å (ϵ = 4300).

Found: C,81.4; H,11.3

Calc. for $C_{32}H_{54}O_2$: C,81.6; H,11.7%.

Lanost-9(11)-enol. - A solution of lanost-9(11)-enyl acetate (100 mg.), from the foregoing experiment, in 3% methanolic potassium hydroxide (30 ml.) was refluxed for 3 hours, cooled and poured into water. The ether extract was washed with water, dried (Na_2SO_4) and evaporated. The residue crystallised from methanol to give lanost-9(11)--enol as matted needles (60 mg.), m.p.166°, $[\alpha]_D$ +73.5° (c, 0.8).

Found: C,83.7; H,12.3

$C_{30}H_{52}O$ requires: C,84.0; H,12.2%.

Hydrolysis, as above, of lanost-9(11)-enyl acetate obtained from cycloartanyl acetate gave an alcohol, m.p.167°, $[\alpha]_D$ +76° (c, 0.8).

Found: C,83.8; H,12.4%.

The melting point was undepressed on mixing with lanost--9(11)-enol described above.

Lanostanyl Acetate (70). - Lanostenyl acetate (200mg.) in glacial acetic acid (150 ml.) was added to platinum catalyst (from 300 mg. platinum oxide) in acetic acid (20 ml.). Shaking with hydrogen was carried out at 80° for 24 hours. The solution was filtered and evaporated under reduced pressure. Crystallisation of the residue from chloroform-methanol gave lanostanyl acetate as fine

needles (160 mg.), m.p.155-156°, $[\alpha]_D$ +41° (c, 1.2). The compound gave no colour with tetranitromethane in chloroform and showed no selective absorption in the ultra-violet.

Found: C,81.4; H,12.1

Calc. for $C_{32}H_{50}O_2$: C,81.3; H,11.9%.

12-Oxolanost-9(11)-enyl Acetate. - Chromic acid (220 mg.) in stabilised acetic acid (50 ml.) was added during 1 hour to a refluxing solution of lanost-9(11)-enyl acetate (500 mg.) in stabilised acetic acid (50 ml.). The solution was heated for a further 2 hours and then kept at room temperature overnight. Methanol was added and the mixture poured into water. The ether extract was washed with 10% sodium carbonate, water and dried (Na_2SO_4). A solution of the product (530 mg.) in light petroleum (100 ml.) was percolated through a column (1.5 cm. x 13 cm.) of alumina (20 g.).

Fraction	Eluant	Volume	Weight	
1 - 3	Light petroleum	150 ml.	-	
4 - 20	Light petroleum: benzene (4:1)	350	162 mg.	Crystalline solid
21 - 22	Benzene	100	30	Gum
23 - 29	''	350	84	Crystalline solid
30 - 34	Benzene:ether(9:1)	250	117	''

Fractions (4-20) (162 mg.) were combined and crystallised from chloroform-methanol to give lanost-9(11)-enyl acetate as hexagonal plates, m.p.170-171° alone and mixed with an authentic sample, $[\alpha]_D$ +84° (c, 1.5). Fractions (23-34) (201 mg.) were combined and crystallised from methanol to give 12-oxolanost-9(11)-enyl acetate as needles, m.p.134-135°, $[\alpha]_D$ +91° (c, 0.6). The melting point was undepressed on mixing with the compound, m.p. 133-135°, isolated from the oxidation of acetate mixture, m.p.137-157° (see above). The compound gave no colour with tetranitromethane in chloroform. Light absorption in ethanol: Max. at 2420 Å (ϵ = 9800).

Found: C,79.3; H,10.9

$C_{32}H_{52}O_3$ requires: C,79.3; H,10.8%.

Hydrogenation of 12-Oxolanost-9(11)-enyl Acetate. - (a) A solution of 12-oxolanost-9(11)-enyl acetate (115mg.) in glacial acetic acid (80 ml.) was shaken with hydrogen at room temperature and atmospheric pressure for 6 hours in presence of platinum catalyst (previously reduced from 100 mg. platinum oxide). The solution was filtered and evaporated under reduced pressure. The residue crystallised from methanol as small plates (60 mg.), m.p.150--155°. Several recrystallisations from the same solvent

gave plates, m.p.166-167°, $[\alpha]_D$ +87° (c, 0.4) which showed a strong yellow colour with tetranitromethane in chloroform. The melting point was undepressed on mixing with a sample of lanost-9(11)-enyl acetate, m.p. 170-171°, $[\alpha]_D$ +87° (c, 0.9).

(b) A solution of 12-oxolanost-9(11)-enyl acetate (100 mg.) in glacial acetic acid (100 ml.) at 30° was shaken with hydrogen over platinum catalyst (from 200 mg. platinum oxide, not previously reduced) for 24 hours. The solution was filtered from platinum and evaporated under reduced pressure. The residue was extracted with light petroleum (40 ml.) and the solution percolated through a column (1 cm. x 12 cm.) of alumina (10 g.).

Fractions	Eluant	Volume	Weight	
1 - 3	Light petroleum	60 ml.	-	
4 - 13	Light petroleum: benzene (9:1)	200	54 mg.	Crystalline solid, m.p.147°
14 - 17	Light petroleum: benzene (7:3)	80	26	Crystalline solid, m.p.151°

Fractions (4-17) were combined and crystallised from chloroform-methanol to give lanostanyl acetate as needles, m.p.156°, $[\alpha]_D$ +40.5° (c, 0.7). The compound gave no colour with tetranitromethane and showed no selective

absorption in the ultra-violet. The melting point was undepressed on mixing with the product, m.p.155-156°, $[\alpha]_D$ +41° (c, 1.2), of hydrogenation of lanost-9(11)-enyl acetate (see above).

Found: C,81.5; H,12.05

Calc. for $C_{32}H_{56}O_2$: C,81.3; H,11.9%.

9:11-Epoxylanostanyl Acetate. - A mixture of lanost--9(11)-enyl acetate (100 mg.) and perhydrol (0.5 ml.) in stabilised acetic acid (10 ml.) was heated on the steam bath for 2 hours. The cooled solution was diluted with water and the crystalline product collected, washed with water, dried and recrystallised from methanol giving 9:11-epoxylanostanyl acetate (60 mg.) as plates, m.p.181--182°, $[\alpha]_D$ +29° (c, 0.7). The compound gives no colour with tetranitromethane in chloroform and shows no selective ultra-violet light absorption.

Found: C,79.2; H,11.4

$C_{32}H_{54}O_3$ requires: C,79.0; H,11.2%.

Lanost-7:9(11)-dienyl Acetate. - 9:11-Epoxylanostanyl acetate (130 mg.) in chloroform (5 ml.) and glacial acetic acid (15 ml.) was treated with 48% hydrobromic acid (10 drops). The solution was kept at room temperature for

5 days and then poured into water. The ether extract was washed with 10% sodium carbonate solution, water and dried (Na_2SO_4). Crystallisation of the product from methanol gave plates (80 mg.), m.p.159-162°. Further crystallisation from methanol gave lanost-7:9(11)--dienyl acetate as plates, m.p.165-166°, $[\alpha]_D$ +87° (c,1.1). The compound showed a red-brown colour with tetranitromethane in chloroform. Light absorption in ethanol: Maxima at 2350 (ϵ = 15,300), 2430 (ϵ = 17,600) and 2520 Å (ϵ = 11,900).

Found: C,81.9; H,11.4

Calc. for $C_{32}H_{52}O_2$: C,82.0; H,11.2%.

Treatment of Lanost-9(11)-enyl Acetate with Hydrogen Chloride. - Lanost-9(11)-enyl acetate (200 mg.) in dry chloroform (25 ml.) at 0° was treated with a vigorous stream of dry hydrogen chloride for 3 hours. The solvent was evaporated under reduced pressure at 30° and the residue crystallised from chloroform-methanol to yield hexagonal plates (170 mg.), m.p.172° alone and mixed with starting material, $[\alpha]_D$ +83° (c, 1.0).

Treatment of cycloArtanyl Acetate with Pyridine Stabilised Iodine Steerate. - (a) cycloArtanyl acetate

(100 mg.) and monopyridine iodine stearate (200 mg., 2 mols, prepared as in 75) were dissolved in dry chloroform (10 ml.) and kept at room temperature for 7 days. After diluting with chloroform, the solution was washed with 10% aqueous sodium thiosulphate, water and dried (Na_2SO_4). The solvent was evaporated and the residue in benzene (100 ml.) was percolated through a column (1.5 cm. x 5 cm.) of alumina (10 g.). Elution with further benzene (200 ml.) gave a fraction (103 mg.) which crystallised from chloroform-methanol as long needles (72 mg.), m.p.130-131° alone and mixed with starting material, $[\alpha]_D$ +53° (c, 1.2).
(b) A solution of cycloartanyl acetate (100 mg.) and monopyridine iodine stearate (200 mg., 2 mols.) in dry chloroform (10 ml.) was refluxed for 2 hours. After cooling, the reaction mixture was worked up exactly as in (a). Evaporation of benzene gave a fraction (105 mg.) which separated from chloroform-methanol as needles (70mg.), m.p.130° undepressed on mixing with starting material, $[\alpha]_D$ +59° (c, 0.8).

Treatment of cycloArtanyl Acetate with t-Butyl Hypochlorite. - (a) A solution of cycloartanyl acetate (250 mg.) in dry carbon tetrachloride (10 ml.) was treated with redistilled t-butyl hypochloride (120 mg., 2 mols.)

and the solution kept in the dark at room temperature for 72 hours. After diluting the reaction mixture with carbon tetrachloride, t-butyl hypochlorite was decomposed by shaking with 10% aqueous potassium iodide acidified with acetic acid. The solution was washed with 10% sodium thiosulphate, water and dried (Na_2SO_4). The product crystallised from chloroform-methanol as needles (237 mg.), m.p.131-132° alone and mixed with starting material, $[\alpha]_D$ +53.3° (c, 0.9).

(b) A solution of cycloartanyl acetate (250 mg.) in dry carbon tetrachloride (10 ml.) was treated with redistilled t-butyl hypochlorite (120 mg., 2 mols.) and the solution refluxed, in the dark, for 4 hours. The reaction mixture was worked up exactly as in (a) above. The product (260 mg.) in light petroleum (50 ml.) was percolated through a column (9 cm. x 1 cm.) of alumina (7 g.). Further elution with light petroleum (350 ml.) gave a fraction (170 mg.) which crystallised from chloroform--methanol as needles, m.p.129-130° undepressed on mixing with starting material, $[\alpha]_D$ +59.2° (c, 0.7). Further elution of the column yielded no crystalline product.

Enol Acetate of cycloArtanone. - A solution of cycloartanone (200 mg.) in isopropenyl acetate (25 ml.)

containing one small drop of concentrated sulphuric acid, was heated on the steam bath for 3 hours. Solid potassium carbonate was added and solvent evaporated under reduced pressure. The ether extract of the residue was washed with 10% aqueous potassium carbonate, water and dried (Na_2SO_4). The product crystallised from methanol as needles (160 mg.), m.p.84-86°. Further crystallisation from methanol gave the enol acetate as needles, m.p.87°, $[\alpha]_D$ +66.5° (c, 1.1). The compound showed a strong yellow colour with tetranitromethane in chloroform. Light absorption in ethanol: Max. at 2060 Å (ϵ = 1590).

Found: C,82.0; H,11.5

$C_{32}H_{52}O_2$ requires: C,82.0; H,11.2%.

Hydrolysis of Enol Acetate of cycloArtanone. - A solution of enol acetate (200 mg.) in 3% methanolic potassium hydroxide (50 ml.) was refluxed for 2 hours. The solution was cooled and poured into water. The ether extract was washed with water and dried (Na_2SO_4). Crystallisation of the product from methanol gave blades (150 mg.), m.p.110° alone or mixed with cycloartanone, $[\alpha]_D$ +22° (c, 2.0). The product showed a pale yellow colour with tetranitromethane and no selective absorption

in the ultra-violet.

19-Deuterolanost-9(11)-enyl Acetate. - cycloArtanyl acetate (1 g.) was dissolved in dry, ethanol free chloroform (30 ml.) in a 250 ml. flask. Deuterium oxide (2 drops, 99.95%) was added and the flask evacuated and filled with deuterium chloride (prepared from re-distilled phosphorus trichloride and 99.95% deuterium oxide; isolated over mercury). The flask was sealed and mechanically shaken for 36 hours. The solvent was evaporated and the residue crystallised from chloroform--methanol as plates (0.93 g.), m.p.140-150°). This material in stabilised acetic acid (75 ml.) was stirred on the steam bath and chromic acid (0.23 g.) in 90% acetic acid added during 5 minutes. Heating was continued for 10 minutes and the mixture poured into water. The ether extract was washed with 10% sodium carbonate, water and dried (Na_2SO_4). A solution of the product (0.96 g.) in light petroleum (100 ml.) was percolated through a column (2 cm. x 12 cm.) of alumina (30 g.). Elution with light petroleum (600 ml.) yielded a fraction (0.45g.) which crystallised from chloroform-methanol as plates, m.p.170-172°. Further crystallisation from the same solvent gave 19-deuterolanost-9(11)-enyl acetate as

plates, m.p.173°, $[\alpha]_D$ +84° (c, 1.2). The melting point was undepressed on mixing with a sample of lanost-9(11)--enyl acetate, m.p.173-174°. The compound showed a strong yellow colour with tetranitromethane in chloroform. Mass spectrographic determination gave deuterium content as 0.815 gram atoms per gram molecule.

Found: C,81.5; H,11.7

$C_{32}H_{54}O_2$ requires: C,81.6; H,11.6%.

19-Deuterolanost-12-oxo-9(11)-enyl Acetate. - A solution of 19-deuterolanost-9(11)-enyl acetate (200 mg.) in Analar acetic acid (30 ml.) was refluxed and chromic acid (200 mg.) in Analar acetic acid (30 ml.) added during 1 hour. Heating was continued for 2 hours and the solution kept at room temperature overnight. Methanol was added and the mixture poured into water. The ether extract was washed with 10% sodium carbonate solution, water and dried (Na_2SO_4). A solution of the product (212 mg.) in light petroleum (25 ml.) was percolated through a column (1 cm. x 10 cm.) of alumina (6 g.). Elution with light petroleum-benzene (1:1, 150 ml.) gave a fraction (130 mg.) which crystallised from methanol as needles (60 mg.), m.p.179-180°. Further crystallisation from methanol gave 19-deuterolanost-12-oxo-9(11)-enyl

acetate as needles, m.p.183° alone or mixed with a sample of 12-oxolanost-9(11)-enyl acetate, $[\alpha]_D$ +90° (c, 0.7). The compound showed no colour with tetranitromethane in chloroform. Light absorption in ethanol: Max. at 2410 Å (ϵ = 9,000). Mass spectrographic determination gave deuterium content as 0.811 gram atoms per gram molecule.

Found: C,79.4; H,10.9

$C_{32}H_{52}O_2$ requires: C,79.3; H,10.8%.

Non-saponifiable Fraction of Opium Marc. - Opium marc (1 kg.) was extracted with boiling chloroform (2x4 l.). The extracts were combined, evaporated to a volume of 4 l. and washed with 3N hydrochloric acid (2 x 3 l.) and water (3 l.). Evaporation of chloroform gave a brown gum (180 g.) which was extracted with a mixture of boiling benzene (300 ml.) and ethanol (2 l.) leaving undissolved a quantity (10 g.) of rubber-like material. Potassium hydroxide (120 g.) in the minimum of water was added to the boiling extract and refluxing continued for 3 hours. The hot solution was then poured into water (10 l.) and the suspension extracted with ether (5 l., 2 x 2.5 l.). The ether extract was washed with water, evaporated, and the residue dried by azeotropic distillation (using benzene) to give an orange gum (43 g.). This non--saponifiable fraction was dissolved in acetone and the solution evaporated to a volume of 100 ml. The solution was kept at room temperature for 4 hours and partly crystalline solid (13.5 g.) separated. Mother liquors were diluted to 100 ml. and kept overnight at room temperature when a further quantity (2.5 g.) of solid separated.

Chromatography of Acetone-crystallised Fraction. - A solution of the combined acetone-crystallised solid (21 g.) in benzene (500 ml.) was percolated through a column (4.5 cm. x 32 cm.) of alumina (520 g.). The column was eluted as shown below.

Fractions	Eluant	Volume	Weight	Description
1 - 8	Benzene	4 l.	1.17 g.	Gum
9 - 20	Benzene:ether (95:5)	6	3.21	White solid
21 - 25	''	2.5	0.92	Gum
26 - 43	'' (90:10)	9	7.11	''
44 - 52	'' (80:20)	4.5	2.85	''
53 - 58	'' (50:50)	3	1.51	''
59 - 67	''	4.5	1.16	''
68 - 70	Benzene:methanol (90:10)	1.5	2.72	Brown gum

Fractions (1-8) and (59-70) did not crystallise readily and have not been further examined.

d-Nonacosan-10-ol. - Fractions (9-20) of the foregoing chromatogram were combined and crystallised from chloroform as plates (1.4 g.), m.p.81-82°. Further crystallisation from ethyl acetate gave d-nonacosan-10-ol as prisms, m.p.81-82°, $[\alpha]_D \pm 0°$ (c, 2.5), showing no

colour with tetranitromethane in chloroform and no selective absorption in the ultra-violet region. The compound gave no colour in the Liebermann-Burchardt test.

Found: C,81.8; H,14.2

Calc. for $C_{29}H_{60}O$: C,82.0; H,14.2%.

cyclo<u>Laudenol.</u> - Fractions (21-58) (above) were combined and crystallised from methanol to yield needles (6.9 g.), m.p.121-123°, $[\alpha]_D$ +46° (c, 1.8). Further crystallisation from methanol gave <u>cyclo</u>laudenol as needles, m.p.123-125°, $[\alpha]_D$ +46° (c, 1.5), showing a pale yellow colour with tetranitromethane in chloroform. Light absorption in ethanol: Max. at 2050 Å (ϵ = 1145).

Found: C,84.6,84.4; H,11.9,12.1

$C_{31}H_{62}O$ requires: C,84.5; H,11.9%.

In the Liebermann-Burchardt test the compound gave a blood red solution having a green fluorescence.

d-<u>Nonacosan</u>-10-<u>yl Acetate.</u> - <u>d</u>-Nonacosan-10-ol (400 mg.) in pyridine (20 ml.) and acetic anhydride (10 mL) was heated in the steam bath for 4 hours. The solution was poured into water and the product collected in ether. The ether extract was washed with 3N hydrochloric acid, water and dried (Na_2SO_4). The solvent was evaporated

and the product crystallised from ethyl acetate-methanol as plates (375 mg.), m.p.44-45°. Several crystallisations from the same solvent gave d-nonacosan-10-yl acetate as rectangular plates, m.p.44.5-45.5°.

Found: C,79.6; H,13.3

Calc. for $C_{31}H_{62}O_2$: C,79.8; H,13.4%.

n-Nonacosan-10-one. - A solution of d-nonacosan-10-ol (500 mg.) in stabilised acetic acid (100 ml.) was stirred at 50° and chromic acid (110 mg.) in acetic acid (25 ml.) added during 30 minutes. Stirring was continued for 1 hour and the mixture kept at room temperature overnight. Methanol was added, the mixture poured into water and the suspension extracted with ether. The ether extract was washed with water and dried (Na_2SO_4). The product crystallised from ethyl acetate as plates (427 mg.), m.p. 74.5-75.5°. By further crystallisation, n-nonacosan--10-one was obtained as plates, m.p.74.5-75.5°.

Found: C,82.4; H,14.0

Calc. for $C_{29}H_{58}O$: C,82.4; H,13.8%.

n-Nonacosane. - Sodium (250 mg.) dissolved in absolute ethanol (15 ml.) was added to n-nonacosan-10-one (225 mg.) and hydrazine hydrate (100%, 2 ml.) and the

mixture heated at 200-210° for 13 hours. The reaction mixture was cooled, poured into water and extracted with ether. The ether extract after being washed with 3N hydrochloric acid, water and dried (Na_2SO_4) was evaporated. Crystallisation from ethyl acetate gave n-nonacosane as plates (186 mg.), m.p.63-64°.

Found: C,84.8; H,14.8

Calc. for $C_{29}H_{60}$: C,85.2; H,14.8%.

cycloLaudenyl Acetate. - cycloLaudenol (250 mg.) in dry pyridine (10 ml.) and acetic anhydride (5 ml.) was kept at room temperature overnight and then poured into water. The ether extract was washed with 3N hydrochloric acid, saturated sodium bicarbonate solution, water and dried (Na_2SO_4). The ether was evaporated and the product crystallised from chloroform-methanol as blades (191 mg.), m.p.118-119°. Further crystallisation from the same solvent gave cyclolaudenyl acetate as blades, m.p.120-121°, $[\alpha]_D$ +55° (c, 1.5, 0.9), showing a plae yellow colour with tetranitromethane in chloroform.

Light absorption in ethanol: Max. at 2060 Å (ϵ = 1500).

Found: C,82.3; H,11.5

$C_{32}H_{54}O_2$ requires: C,82.1; H,11.3%.

cyclo*Laudenyl Benzoate.* *cyclo*Laudenol (400 mg.) in dry pyridine (5 ml.) and redistilled benzoyl chloride (1 ml.) was refluxed for 1.5 hours. The solution was cooled, poured into water and the suspension extracted with ether. The extract was washed with 3N hydrochloric acid, 5% sodium hydroxide solution, water and dried (Na_2SO_4). The ether was evaporated and the product crystallised from chloroform-methanol to give needles (414 mg.), m.p.190-192°. Several crystallisations from the same solvent yielded cyclo*laudenyl benzoate* as needles, m.p.194-195°, $[\alpha]_D$ +63° (c, 0.8, 0.9) showing a pale yellow colour with tetranitromethane.

Found: C,83.5; H,10.5

$C_{38}H_{56}O_2$ requires: C,83.8; H,10.4%.

The benzoate (200 mg.) in 5% ethanolic potassium hydroxide solution (100 ml.) was refluxed for 3 hours. The solution was poured into water and the product collected in ether. Crystallisation from methanol yielded needles (110 mg.), m.p.125° alone and mixed with *cyclo*laudenol, $[\alpha]_D$ +45° (c, 1.0).

cyclo*Laudenone.* - A solution of *cyclo*laudenol (460 mg.) in stabilised acetic acid (100 ml.) at room temperature was treated with chromic acid (95 mg.) in

acetic acid (20 ml.) during 30 minutes. The solution was kept overnight at room temperature and then methanol added. The mixture was poured into water and the product collected in ether. After being washed with 5% sodium carbonate solution, water and dried (Na_2SO_4) the ether solution was evaporated. The residue was crystallised from methanol to give blades (285 mg.), m.p.112--114°. A second crop of blades (47 mg.), m.p.111°, was obtained from the mother liquor. The combined material was several times crystallised from methanol to yield cyclolaudenone as blades, m.p.115°, $[\alpha]_D$ +19° (c, 1.3), showing a pale yellow colour with tetranitromethane in chloroform.

Found: C,84.3; H,11.6

$C_{31}H_{50}O$ requires: C,84.4; H,11.8%.

cycloLaudanyl Acetate. - cycloLaudenyl acetate (500 mg.) in glacial acetic acid (150 ml.) was shaken, at atmospheric pressure and temperature, with hydrogen in presence of platinum catalyst (previously reduced from 300 mg. platinum oxide). After 30 minutes absorption of hydrogen ceased and the solution was filtered from platinum and evaporated under reduced pressure. Crystallisation from chloroform-methanol yielded needles

(440 mg.), m.p.131-133°. Further crystallisation from the same solvent gave cyclo*laudanyl acetate* as needles, m.p.132-133°, $[\alpha]_D$ +50° (c, 0.8, 0.7) showing a pale yellow colour with tetranitromethane and no selective ultra-violet light absorption.

Found: C,81.8; H,11.7

$C_{33}H_{56}O_2$ requires: C,81.75; H,11.6%.

cyclo*Laudanol.* - A solution of *cyclo*laudanyl acetate (180 mg.) in 3% ethanolic potassium hydroxide (100 ml.) was refluxed for 3 hours, cooled and poured into water. The ether extract was washed with water, dried (Na_2SO_4) and evaporated. The product crystallised from methanol as needles (115 mg.), m.p.133-134°. Several crystallisations from methanol gave cyclo*laudanol* as needles, m.p. 133-134°, $[\alpha]_D$ +43° (c, 0.9, 0.8).

Found: C,83.9; H,12.1

$C_{31}H_{54}O$ requires: C,84.1; H,12.3%.

cyclo*Laudenyl Acetate Oxide.* - To *cyclo*laudenyl acetate (500 mg.) in chloroform (10 ml.) at 0° was added a chloroform solution of perbenzoic acid (1.7 ml., 106 mg. ml., 1.2 mol.). The mixture was kept at 0° for 24 hours and then a sample (2 ml.) withdrawn and titrated

with standard sodium thiosulphate solution. Comparison with a blank experiment showed reaction of acetate with 0.98 mol. of perbenzoic acid. The remainder of the reaction mixture was diluted with chloroform and washed with 10% aqueous sodium thiosulphate, 10% aqueous sodium hydroxide, water and dried (Na_2SO_4). The solvent was evaporated and the residue crystallised from methanol as needles (336 mg.), m.p.150-151°. Further crystallisation gave cyclolaudenyl acetate oxide as needles, m.p.153-154°, $[\alpha]_D$ +54° (c, 1.2, 1.8) showing a pale yellow colour with tetranitromethane and no selective absorption in the ultra-violet.

Found: C,79.0; H,10.9

$C_{33}H_{54}O_3$ requires: C,79.5; H,10.9%.

cyclolaudenyl Acetate Dibromide. - A solution of bromine in acetic acid (7.5 ml., 15.1 mg./ml., 1.1 mol.) was added to cyclolaudenyl acetate (300 mg.) in acetic acid at room temperature. The product crystallised from the reaction mixture as needles (210 mg.), m.p.170-171° (decomp.). Several crystallisations from chloroform-methanol gave cyclolaudenyl acetate dibromide as needles, m.p.179-180° (decomp.), $[\alpha]_D$ +33° (c, 1.0, 0.8, 0.7).

The compound gave a pale yellow colour with tetranitromethane and showed no selective ultra-violet light absorption.

Found: C,62.7; H,8.7

$C_{33}H_{54}O_2Br_2$ requires: C,61.7; H,8.5%.

Laudanyl Acetate. - A solution of cyclolaudanyl acetate (1 g.) in dry chloroform (50 ml.) at 0° was treated with a vigorous stream of dry hydrogen chloride during 3 hours. The solvent was then evaporated under reduced pressure and the crude residue dissolved in glacial acetic acid (100 ml.). The solution was shaken with hydrogen at 80° for 7 hours in presence of platinum catalyst (from 500 mg. platinum oxide not previously reduced). After filtration from platinum, the solution was evaporated under reduced pressure and the residue crystallised from chloroform-methanol as needles (690 mg.), m.p.153-156°, $[\alpha]_D$ +35° (c, 1.3) showing a yellow colour with tetranitromethane. A solution of this material (690 mg.) in stabilised acetic acid (100 ml.) was stirred on the steam bath and chromic acid (400 mg.) in acetic acid (25 ml.) was added during 30 minutes. The solution was heated for 1 hour, cooled, methanol was added and the

mixture poured into water. The ether extract was washed with 2N hydrochloric acid, 5% aqueous sodium hydroxide, water and dried (Na_2SO_4). A solution of the yellow product (670 mg.) in light petroleum (50 ml.) was percolated through a column (1.5 cm. x 12 cm.) of alumina (20 g.).

Fractions (2-10) (280 mg.) eluted by light petroleum (500 ml.) crystallised from chloroform-methanol as needles (195 mg.), m.p.169-171°. Further crystallisation gave laudanyl acetate as needles, m.p.171°, $[\alpha]_D$ +32° (c, 1.2, 1.1). The compound showed no colour with tetranitromethane and no selective absorption in the ultra-violet.

Found: C,81.2; H,12.3

$C_{33}H_{53}O_3$ requires: C,81.4; H,12.0%.

Fractions (11-12) (280 mg.) eluted by methanol (100 ml.) were combined with the corresponding fractions (640 mg.) obtained by chromatography of the product (2.2 g.) of a repeat experiment. The combined material (920 mg.) dissolved in pyridine (20 ml.) and acetic anhydride (10 ml.), was heated on the steam bath for 3 hours. After working up in the usual way a solution of the product (790 mg.) in light petroleum (50 ml.) was percolated through a column (1.5 cm. x 14 cm.) of alumina (25 g.). Fractions

(1-6) (250 mg.) eluted by light petroleum (300 ml.) crystallised from chloroform-methanol as needles (152 mg.) m.p.170-171° alone and mixed with laudanyl acetate, $[\alpha]_D$ +34° (c, 1.2).

Fractions (8-10) (170 mg.) eluted by light petroleum--benzene (80:20, 150 ml.) crystallised from methanol as pale yellow needles, m.p.183-184° alone and mixed with 7:11-dioxolaud-8-enyl acetate (see below), $[\alpha]_D$ +67.5° (c, 0.6).

Light absorption in ethanol: Max. at 2720 Å (ϵ = 6900).

Further elution of the column gave no homogeneous product.

Laudanol. - A solution of laudanyl acetate (78 mg.) in 3% methanolic potassium hydroxide solution (50 ml.) was refluxed for 3 hours, cooled and poured into water. The ether extract was washed with water, dried (Na_2SO_4) and evaporated. The residue crystallised from chloroform--methanol to give laudanol as fine needles (65 mg.), m.p. 200-201°, $[\alpha]_D$ +21° (c, 0.9).

Found: C,84.0; H,12.8

$C_{31}H_{56}O$ requires: C,83.7; H,12.7%.

Laudanone. - A solution of chromic acid (100 mg.) in stabilised acetic acid (20 ml.) was added, with stirring, during 30 minutes to a solution of laudanol (500 mg.) in

Analar benzene (20 ml.) and acetic acid (100 ml.) at 40°. The solution was kept at room temperature overnight, methanol added and the mixture poured into water. The ether extract was washed with 10% sodium carbonate solution, water and dried (Na_2SO_4). The ether was evaporated and the residue crystallised from chloroform--methanol to give laudanone as small plates (360 mg.), m.p.131-132°, $[\alpha]_D$ +14° (c, 0.8, 1.0).

Found: C,84.1; H,12.5

$C_{31}H_{54}O$ requires: C,84.1; H,12.3%.

Laudane. - Laudanone (200 mg.) with hydrazine hydrate (100%, 2 ml.) and a solution of sodium (250 mg.) in absolute ethanol (10 ml.) was heated at 200° for 18 hours. The reaction mixture was cooled and poured into water. The suspension was extracted with ether and the extract washed with 3N hydrochloric acid, water and dried (Na_2SO_4). A solution of the product in light petroleum (25 ml.) was filtered through a column (1 cm. x 9 cm.) of alumina (6 g.). Elution with light petroleum (50 ml.) yielded a fraction (130 mg.) which crystallised from chloroform-methanol to give laudane as plates (145 mg.), m.p.142-143°, $[\alpha]_D$ +25° (c, 1.6, 1.7) showing no colour with tetranitromethane in chloroform and no absorption in

the ultra-violet.

Found: C,87.0; H,13.2

$C_{31}H_{56}$ requires: C,86.8; H,13.2%.

Laudenyl Acetate. - (a) A solution of cyclolaudanyl acetate (1 g.) in dry chloroform (50 ml.) at 0° was treated with a vigorous stream of dry hydrogen chloride for 3 hours. The solvent was evaporated under reduced pressure and the product crystallised from chloroform--methanol as needles (770 mg.), m.p.145-155°, $[\alpha]_D$ +62° (c, 1.5) showing a strong yellow colour with tetranitromethane. Light absorption in ethanol: Max. at 2080 Å (ϵ = 3100). Six recrystallisations from chloroform--methanol gave laudenyl acetate as needles (30 mg.), m.p. 173-174°, $[\alpha]_D$ +81° (c, 0.9). Light absorption in ethanol: Max. at 2060 Å (ϵ = 3100).

Found: C,81.4; H,11.5.

$C_{33}H_{56}O_2$ requires: C,81.75; H,11.6%.

(b) A solution of cyclolaudanyl acetate (1.8 g.) in dry chloroform (50 ml.) was treated with hydrogen chloride as in (a) to give needles (1.47 g.), m.p.145-155°. Chromic acid (440 mg.) in 90% acetic acid (25 ml.) was added, with stirring, during 5 minutes to a solution of this material (1.47 g.) in stabilised acetic acid (100 ml.) heated on

the steam bath. The mixture was heated for 10 minutes, poured into water and the product extracted with ether. The ether extract was washed with 10% sodium carbonate solution, water and dried (Na_2SO_4). A solution of the yellow product (1.46 g.) in light petroleum (100 ml.) was percolated through a column (2 cm. x 16 cm.) of alumina (45 g.).

Fractions	Eluant	Volume	Weight	Description	M.p.
1 - 11	Light petroleum	1100ml.	610mg.	White solid	171-173°
12 - 24	Light petroleum: benzene (80:20)	1300	300	Gum	
25 - 30	Light petroleum: benzene (50:50)	600	270	Yellow crystals	184-185
31 - 32	Benzene	200	67	Gum	
33 - 37	''	500	120	White solid	188-189

Fractions (1-11) crystallised from chloroform-methanol as blades (511 mg.), m.p.171-173°. Further crystallisation from the same solvent gave blades, m.p.173° alone and mixed with laudenyl acetate from (a), $[\alpha]_D$ +80° (c, 1.4). Light absorption in ethanol: Max. at 2060 Å (ϵ = 3200).

Found: C,81.8: H,11.9

Calc. for $C_{33}H_{56}O_2$: C,81.75; H,11.6%.

Fractions (25-30) were combined and crystallised from methanol as light yellow blades (175 mg.), m.p.183-184°:

Further crystallisation yielded 7:11-dioxolaud-8-enyl acetate as light yellow blades, m.p.186°, $[\alpha]_D$ +71° (c, 1.1).

Light absorption in ethanol: Max. at 2700 Å (ϵ = 7400).

Found: C,77.0; H,10.5

$C_{33}H_{42}O_4$ requires: C,77.3; H,10.2%.

Fractions (33-37) were combined and crystallised from methanol as needles (50 mg.), m.p.189-191°. Recrystallisation from methanol gave blades, m.p.192-193° alone and mixed with 12-oxolaud-9(11)-enyl acetate (see below) $[\alpha]_D$ +85° (c, 1.4).

Light absorption in ethanol: Max. at 2410 Å (ϵ = 11,400).

12-Oxolaud-9(11)-enyl Acetate. - Chromic acid (300mg.) in Analar acetic acid (50 ml.) was added during 1 hour to a refluxing solution of laudenyl acetate (300 mg.) in Analar acetic acid (50 ml.). Heating was continued for 2 hours and then methanol added and the mixture poured into water. The ether extract was washed with 10% sodium carbonate solution, water and dried (Na_2SO_4). A solution of the product (283 mg.) in light petroleum (25 ml.) was percolated through a column (1 cm. x 14 cm.) of alumina (9 g.).

Fractions (2 - 6) (37 mg.), eluted by light petroleum

(125 ml.), crystallised from chloroform-methanol as blades (30 mg.), m.p.171-172° alone and mixed with laudenyl acetate, showing a strong yellow colour with tetranitromethane.

Fractions (10-21) (130 mg.), eluted by benzene-light petroleum (50:50, 150 ml.), benzene (75 ml.) and benzene--ether (30:20, 75 ml.), crystallised from methanol as blades (100 mg.), m.p.192-193°. Several recrystallisations from methanol gave 12-oxolaud-9(11)-enyl acetate as blades, m.p.194°, $[\alpha]_D$ +87° (c, 0.9, 0.8), showing no colour with tetranitromethane in chloroform.

Light absorption in ethanol: Max. at 2410 Å (ϵ = 10,300).

Found: C,80.0; H,10.9

$C_{33}H_{54}O_3$ requires: C,79.5; H,10.9%.

7:11-Dioxolaudanyl Acetate. - Zinc dust (1 g.) was added in portions to a refluxing solution of 7:11-dioxolaud-8-enyl acetate (50 mg.) in stabilised acetic acid (10 ml.). Refluxing was continued for 1 hour and then the solution was decanted from zinc which was further extracted with boiling acetic acid (10 ml.). The acetic acid solutions were combined and poured into water. The ether extract was washed with 10% sodium carbonate solution, water and dried (Na_2SO_4). The ether was then

evaporated and the residue crystallised from methanol as plates (34 mg.), m.p.241-242°. Recrystallisation gave 7:11-dioxolaudanyl acetate as plates, m.p.241°, $[\alpha]_D$ +50.5° (c, 1.5) showing no colour with tetranitromethane and no selective absorption in the ultra-violet.

Found: C,77.2; H,10.7

$C_{33}H_{54}O_4$ requires: C,77.0; H,10.6%.

trisnorcycloArtan-24-aldehyde Acetate. - A solution of cycloartenyl acetate (4 g.) in dry chloroform (200 ml.) at -45° was treated with ozonised oxygen (10.4 ml. O_3/min., 2 mols.) for 40 minutes. After the solution had warmed to room temperature, glacial acetic acid (50 ml.) was added. Zinc dust (4 g.) was added during 30 minutes, the solution being stirred and the temperature kept below 20°. After stirring for a further 30 minutes, the chloroform solution was filtered and washed with saturated aqueous sodium bicarbonate, water and dried (Na_2SO_4). The solvent was evaporated and the residue crystallised from aqueous acetone as prisms (2.33 g.), m.p.155-160° (decomp.). Recrystallisation from the same solvent gave trisnorcyclo-artan-24-aldehyde acetate as prisms, m.p.155-157° (decomp.), $[\alpha]_D$ +59.5° (c, 2.0) showing a pale yellow colour with tetranitromethane and no selective absorption in the

ultra-violet.

Found: C,78.7; H,10.5

$C_{29}H_{46}O_3$ requires: C,78.7; H,10.5%.

Attempted crystallisation of trisnorcycloartan-24-aldehyde acetate from aqueous methanol gave 24:24-dimethoxytrisnorcycloartanyl acetate as prisms, m.p.125-126° depressed to 115° on mixing with starting material, $[\alpha]_D$ +53° (c, 1.1)

Found: C,76.0; H,10.7

$C_{31}H_{52}O_4$ requires: C,76.2; H,10.7%.

To a refluxing solution of trisnorcycloartan-24-aldehyde acetate (250 mg.) in methanol (20 ml.) was added hydroxylamine hydrochloride (300 mg.) and sodium acetate (500 mg.) in water (1 ml.) and methanol (15 ml.). Refluxing was continued for 2 hours and then the reaction mixture was poured into water and the product collected in ether in the usual way. Crystallisation from methanol gave the oxime as needles (190 mg.), m.p.193°.

Found: C,75.7; H,10.1

$C_{29}H_{47}O_3N$ requires: C,76.1; H,10.3%.

24-Oxobisnorcycloartanyl Acetate. - A solution of trisnorcycloartan-24-aldehyde acetate (1.4 g.) in dry ether (20 ml.) and dioxan (10 ml.) was treated with a dry ethereal solution (50 ml.) of diazomethane (from 5 g.

N-nitrosomethyl urea). The reaction mixture was kept at room temperature for 3 days and the solvent was evaporated under reduced pressure. A solution of the product (1.5 g.) in light petroleum-benzene (80:20, 150 ml.) was percolated through a column (2.5 cm. x 17 cm.) of alumina (60 g.).

Fractions (5-22) (850 mg.), eluted by light petroleum--benzene (60:40, 1200 ml.; 40:60, 600 ml.), were combined and crystallised from methanol as plates (670 mg.), m.p. 168-170°. Further crystallisation gave 24-oxobisnorcycloartanyl acetate as plates, m.p.170°, $[\alpha]_D$ +58° (c, 1.2) showing a pale yellow colour with tetranitromethane and no selective ultra-violet light absorption.

Found: C,78.9; H,10.6

$C_{30}H_{48}O_3$ requires: C,78.9; H,10.6%.

To a refluxing solution of 24-oxobisnorcycloartanyl acetate (100 mg.) in methanol (15 ml.) was added hydroxylamine hydrochloride (200 mg.) and sodium acetate (400 mg.) in water (1 ml.) and methanol (10 ml.). Refluxing was continued for 2.5 hours and then the reaction mixture was poured into water and the product isolated in the usual way. Crystallisation from chloroform-methanol gave the oxime as plates (35 mg.), m.p.219-220°.

Found: C,76.2; H,10.0

$C_{30}H_{49}O_3N$ requires: C,76.4; H,10.5%.

Chromic acid (20 g.) in stabilised acetic acid (10 ml.) was added during 30 minutes to 24-oxobisnorcycloartanyl acetate (100 mg.) in acetic acid (25 ml.) at room temperature. The solution was kept overnight at room temperature but showed no colour change to indicate reaction. Methanol was added to the mixture which was then poured into water and the product collected in ether in the usual way. Crystallisation from methanol gave plates (72 mg.), m.p.163-169° alone and mixed with starting material, $[\alpha]_D$ +58° (c, 1.0).

24-Oxonorcycloartanyl Acetate. - A solution of trisnorcycloartan-24-aldehyde acetate (2.8 g.) in dry ether (30 ml.) and dioxan (20 ml.) was treated with a dry ether solution (120 ml.) of diazoethane (from 12 g. N-nitrosoethyl urea). The reaction mixture was kept at room temperature for 2 days and then evaporated under reduced pressure to give a gum which was dissolved in light petroleum (100 ml.) and filtered through a column (18 cm. x 2.5cm.) of alumina (90 g.).

Fractions (5-7) (650 mg.), eluted by light petroleum--benzene (30:20, 450 ml.), separated from aqueous methanol

as mixed crystals, m.p.95-100° which yielded no homogeneous compound.

Fractions (8-23) (1.19 g.), eluted by light petroleum--benzene (80:20, 1050 ml.; 50:50, 1350 ml.), crystallised from aqueous methanol as blades (1 g.), m.p.116-117°. Recrystallisation from aqueous methanol gave 24-oxonorcycloartanyl acetate as blades, m.p.118°, $[\alpha]_D$ +57° (c,1.4) showing a pale yellow colour with tetranitromethane and no selective absorption in the ultra-violet.

Found: C,78.9; H,10.8

$C_{31}H_{50}O_3$ requires: C,79.1; H,10.7%.

Hydroxylamine hydrochloride (300 mg.) and sodium acetate (500 mg.) in water (1 ml.) and methanol (15 ml.) were added to a refluxing solution of 24-oxonorcycloartanyl acetate (200 mg.) in methanol (20 ml.). The mixture was refluxed for 3 hours, poured into water and the product isolated in the usual way. Crystallisation from methanol gave the oxime as needles (140 mg.), m.p.160°.

Found: C,77.2; H,10.4

$C_{31}H_{51}O_3N$ requires : C,76.7; H,10.6%.

24-Oxonorcycloartanyl acetate (100 mg.) in stabilised acetic acid (20 ml.) was treated with chromic acid (20 mg.) in acetic acid (10 ml.) exactly as for 24-oxobisnorcyclo-

artanyl acetate (see above). The product crystallised from methanol as blades (70 mg.), m.p.115-117° alone and mixed with starting material, $[\alpha]_D$ +57° (c, 1.1).

24-Methylcycloartanyl Acetate. - To a stirred suspension of magnesium (140 mg., 6 g. atoms) in dry ether (10 ml.) was added isopropyl bromide (700 mg., 6 mols.) in ether (20 ml.), during 30 minutes. After being stirred and refluxed for 1 hour the solution was cooled. 24-Oxobisnorcycloartanyl acetate (430 mg., 1 mol.) in ether (50 ml.) was added during 30 minutes to the stirred Grignard solution and then the mixture was refluxed for 2 hours. The reaction mixture was diluted with ether, added to 3N hydrochloric acid and the ether layer washed with 3N hydrochloric acid, water and dried (Na_2SO_4). The ether was evaporated, the residue in pyridine (10 ml.) and acetic anhydride (10 ml.) kept at room temperature overnight and the reaction mixture worked up in the usual way to give white gummy solid (450 mg.). This material, without further purification, was dissolved in pyridine (40 ml.) and phosphorus oxychloride (5 ml.) and heated on the steam bath for 3 hours. After pouring the cooled solution into water, the product was collected in ether. The extract was washed with 3N hydrochloric acid, 10%

aqueous sodium bicarbonate, water and dried (Na_2SO_4). The ether was evaporated and a solution of the brown residual gum (200 mg.) in light petroleum (25 ml.) was percolated through a column (1 cm. x 3 cm.) of alumina (6 g.). Elution with light petroleum (150 ml.) gave a gum (50 mg.) which crystallised from methanol as needles, m.p.130-150°, showing a strong yellow colour with tetranitromethane. Further elution of the column gave no crystallisable product.

A solution of the material, m.p.130-150° (50 mg.), in glacial acetic acid (70 ml.) was shaken with hydrogen and platinum catalyst (previously reduced from 50 mg. platinum oxide) for 3 hours. The solution was filtered from platinum and evaporated under reduced pressure. The product crystallised from methanol as blades (32 mg.), m.p.122-123°. Further crystallisation from methanol gave 24-methylcycloartanyl acetate as blades, m.p.123°, $[\alpha]_D$ +59.5° (c, 1.4). Although this compound differs from cyclolaudanyl acetate in melting point, optical rotation and crystalline form, it showed no melting point depression when mixed with a sample of cyclolaudanyl acetate, m.p.132°, $[\alpha]_D$ +60° (c, 0.8).

Found: C,81.9; H,11.8

$C_{33}H_{56}O_2$ requires: C,81.8; H,11.6%.

The compound gave a pale yellow colour with tetranitromethane in chloroform and showed no selective ultra-violet absorption.

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